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ORNAMENTAL FLOWERING PLANTS EXPERIMENTALLY INFECTED WITH CURLY TOP[1]

JULIUS H. FREITAG[2] AND HENRY H. P. SEVERIN[3]

(Contribution from the Division of Entomology and Parasitology, College of Agriculture, University of California, coöperating with the United States Department of Agriculture Bureau of Entomology.)

INTRODUCTION

SEVERAL INVESTIGATORS have recorded the experimental transmission of virus diseases of plants to a large number of species in different genera of many families. A review of the literature on the host range of certain virus diseases shows an extensive host range as determined by experimental infection, but the reported natural host range is often limited, as illustrated in table 1.

The natural infection of ornamental flowering plants with curly top has already been reported in a previous paper.(27)[4] A list of the plants experimentally infected, but without details of the experiments or description of the symptoms, was published in the *Plant Disease Reporter*.(7) The details and results of the experiments performed to experimentally infect ornamental flowering plants with curly top are given in the present paper. No intensive investigations have been conducted to determine which plants are resistant or immune to curly top; only plants experimentally infected with the disease are reported in this paper. The symptoms on the susceptible host plants are briefly described. The longevity of the last living male and female beet leafhopper, *Eutettix tenellus* (Baker), was ascertained on inoculated plants and a record was kept of all plants on which the leafhopper completed its life cycle.

[1] Received for publication February 14, 1936.

[2] Junior Entomologist in the Experiment Station.

[3] Associate Entomologist in the Experiment Station.

[4] Superscript numbers in parentheses refer to "Literature Cited" at the end of the paper.

METHODS OF EXPERIMENTALLY INFECTING PLANTS WITH CURLY TOP

The methods used in the transmission of curly top to ornamental flowering plants were somewhat similar to those described in the experimental infection of economic plants and weeds reported in an earlier contribution.[28] The ornamental flowering plants were grown from seeds in the greenhouse and were fumigated with Nico-Fume tobacco-paper insecticide prior to using. Each plant was enclosed in a cage and inoculated with curly top by either infective male or female beet leafhoppers which had completed their nymphal stages on beets in an advanced stage of t' e disease. The number of insects used varied from 5 to 20, according to the size of the plants. When the longevity of the adults was short, presumably because of unfavorable food, the plants were repeatedly inoculated by different lots of infective insects.

TABLE 1

Host Ranges of Some Plant Virus Diseases as Determined by Natural and Experimental Infection

Virus disease	Authority*	Locality	Plants experimentally infected			Plants naturally infected		
			Species	Genera	Families	Species	Genera	Families
Sugar beet curly top	Severin and co-workers (7, 14, 15, 17, 18, 19, 20, 25, 26, 27, 28)	California	194	122	40	75	48	18
	Carsner (6)	California	14	13	8	..	..	..
Aster yellows	Kunkel (11, 12)	New York	170	150	38	9	9	4
	Severin (21, 24, 26, 27)	California	7	5	2	14	13	6
Southern celery mosaic	Wellman (33, 34)	Florida	81	59	23	42	35	17
Tobacco ring-spot	Wingard (35, 36)	Virginia	62	38	17	11	9	3
Tomato spotted wilt	Bald and Samuel (5)	Australia	35	15	4	4	4	4
	Smith (29, 30, 31)	England	28	13	4	4	4	4
	Gardner and co-workers (8, 9, 32)	California	30	24	19	31	31	17
Tobacco mosaic	Grant (10)	Wisconsin	29	24	14	..	..	..
Tomato bunchy top	McClean (13)	South Africa	15	8	2	..	..	..

* Numbers in parentheses refer to numbers in list of "Literature Cited" at the end of this paper.

The plants were exposed to the infective leafhoppers until symptoms of curly top developed, which usually required about two weeks; if no symptoms appeared, the insects were kept on the plants for a period of 6 weeks. After inoculation the cage containing the infective specimens was removed, and care was taken to see that the plant was free from all insects.

Healthy plants of each species or variety were exposed to noninfective beet leafhoppers as a check, and as a further check, healthy plants were grown in cages free from insects.

No species or variety of ornamental flowering plant inoculated with curly top was considered to be infected with the disease on the basis of symptoms, unless the virus was recovered by previously noninfective male leafhoppers or nymphs and transferred to sugar beets. From 2 to 10 plants of each species or variety were inoculated, as indicated in table 2. Small plants were ordinarily used, but sometimes plants susceptible to curly top would die before the virus could be transferred to sugar beets, and hence larger plants were inoculated.

In one method of recovering the virus, the inoculated plants were exposed to previously noninfective male leafhoppers enclosed in a cage for a period of 2 or 3 days, or longer if the food material was favorable. (In order to avoid egg deposition, females were not used in this type of recovery tests.) The insects were then transferred from the inoculated plant to two healthy beet seedlings, each enclosed in a cage. Two beet seedlings were used in each test because occasionally a seedling is killed by damping-off. The leafhoppers were confined in cages enclosing the beet seedlings for a period of 5 to 7 days, and then the cages containing the leafhoppers were removed.

In another method of recovering the virus, nymphs which hatched from eggs deposited in favorable food plants by infective females used to inoculate the plants, were transferred to beet seedlings. It has been established [16, 23] that nymphs which hatch from eggs deposited by infective females are not able to transmit curly top unless the nymphs have previously fed on a diseased plant.

The inoculated beets were fumigated with Nico-Fume tobacco-paper insecticide and were kept within insect-proof cages for a period of 3 months, if symptoms of curly top did not develop within the usual period of 1 to 2 weeks. If the beets developed curly top, it is evident that the inoculated ornamental flowering plant had been infected with the disease.

TABLE 2

LIST OF ORNAMENTAL FLOWERING PLANTS EXPERIMENTALLY INFECTED WITH CURLY TOP, LONGEVITY, AND LIFE CYCLE* OF BEET LEAFHOPPER†

Family	Name of plant: Common	Name of plant: Scientific	Seasons duration	Number of plants: Inoculated	Number of plants: Virus recovered	Longevity, days: Males	Longevity, days: Females
Moraceae	Japanese hop	*Humulus japonicus* Sieb. and Zucc.	Perennial grown as annual	3	3	16	14–18
Chenopodiaceae	Common summer-cypress	*Kochia trichophylla* Stapf	Annual	3	3	..	C*
Amaranthaceae	.....	*Amaranthus aurora*	Annual	3	3	..	C
	Josephs-coat	*Amaranthus gangeticus* L.	Annual	3	3	..	C
	Love-lies-bleeding	*Amaranthus caudatus* L.	Annual	3	3	..	C
	Feather cockscomb	*Celosia argentea* L.	Annual	8	8	..	C
	Common globe-amaranth	*Gomphrena globosa* L.	Annual	6	6	..	C
Nyctaginaceae	Common four-o'clock	*Mirabilis jalapa* L.	Perennial grown as annual	7	5	..	C
Portulacaceae	Common portulaca	*Portulaca grandiflora* Hook.	Annual	2	2	..	C
	Common rockpurslane	*Calandrinia grandiflora* Lindl.	Perennial	6	2	..	C
Caryophyllaceae	Sweet william	*Dianthus barbatus* L.	Perennial	6	6	..	C
	Chinese pink	*Dianthus chinensis* L.	Annual	5	3	..	C
	Heddewig pink	*Dianthus chinensis* L. var. *heddewigii* Regel	Perennial grown as annual	7	6	..	C
	Grass pink	*Dianthus plumarius* L.	Perennial	7	3	3–12	12–27
	Carnation	*Dianthus caryophyllus* L.	Perennial	7	2	..	C
	Babysbreath	*Gypsophila paniculata* L.	Perennial	5	3	..	C
	Drooping catchfly	*Silene pendula* L.	Annual	5	4	..	C
	Maltese cross	*Lychnis chalcedonica* L.	Perennial	4	3	..	C
	Haage campion	*Lychnis haageana* Lem.	Perennial	6	5	6–9	48
Ranunculaceae	Poppy anemone	*Anemone coronaria* L.	Perennial	5	5	..	C
	Love-in-a-mist	*Nigella damascena* L.	Annual	6	6	..	C
	Orange larkspur	*Delphinium nudicaule* Torr. & Gray	Perennial	2	2	..	C
Papaveraceae	Iceland poppy	*Papaver nudicaule* L.	Perennial	7	6	..	.....
			Perennial	7	3	7–12	16–36
	Oriental poppy; Salmon Queen variety, Oriental poppy	*Papaver orientale* L.	Perennial	7	5	6–25	22–77
	Oriental poppy hybrid		Perennial	7	2	20–30	54

* C indicates that the beet leafhopper completed its life cycle on host plant.

† The common names of the ornamental flowering plants were obtained from *Standardized Plant Names* (1), or if the common names were not given in this publication, then from Bailey (2, 3) or L. H. Bailey and E. Z. Bailey (4).

TABLE 2—(*Continued*)

Family	Name of plant: Common	Name of plant: Scientific	Seasons duration	Number of plants: Inoculated	Number of plants: Virus recovered	Longevity, days: Males	Longevity, days: Females
Capparidaceae	Spiderflower	*Cleome spinosa* Jacq.	Annual	6	5	..	C*
Cruciferae	Annual stock	*Matthiola incana* R. Br. var. *annua* Voss	Annual	4	3	..	C
	Dames rocket	*Hesperis matronalis* L.	Biennial or perennial	3	3	..	C
	Honesty	*Lunaria annua* L.	Annual or biennial	5	5	..	C
	Purple candytuft	*Iberis umbellata* L.	Annual	6	3	..	C
Resedaceae	Common mignonette	*Reseda odorata* L.	Annual	6	6	..	C
Geraniaceae	Fish geranium	*Pelargonium hortorum* Bailey	Perennial	3	2	5–16	8–16
Tropaeolaceae	Canary nasturtium	*Tropaeolum peregrinum* L.	Annual	2	2	..	C
Euphorbiaceae	Snow-on-the-mountain	*Euphorbia marginata* Pursh.	Annual	5	3	4–8	11–18
Sapindaceae	Balloonvine	*Cardiospermum halicacabum* L.	Perennial grown as annual	2	2	..	C
Malvaceae	Herb treemallow	*Lavatera trimestris* L.	Annual	2	2	..	C
Violaceae	Tufted pansy	*Viola cornuta* L.	Perennial	4	4	..	C
Onagraceae	Clarkia	*Clarkia elegans* Dougl.	Annual	4	4	..	C
Umbelliferae	Blue laceflower	*Trachymene caerulea* R. Graham	Annual	5	4	24	.....
Primulaceae	Top primrose	*Primula obconica* Hance	Perennial	1	1	..	C
	....	*Primula saxatalis* Komar	Perennial	2	2	..	C
	Cowslip primrose	*Primula veris* L.	Perennial	3	3	..	C
	Oxlip primrose	*Primula elatior* Hill	Perennial	4	4	..	C
Plumbaginaceae	Notchleaf sea-lavender	*Limonium sinuatum* Mill.	Biennial or perennial	6	4	..	C
Apocynaceae	Madagascar periwinkle	*Vinca rosea* L.	Perennial grown as annual	6	5	36–75	50–92
Convolvulaceae	Crimson starglory	*Quamoclit lobata* House	Perennial	4	1	5–20	11–24
	Brazilian morning-glory	*Ipomoea setosa* Ker.	Perennial	2	2	..	C
Polemoniaceae	Drummond phlox	*Phlox drummondii* Hook	Annual	3	3	20–40	43
	Purplebell cobaea	*Cobaea scandens* Cav.	Perennial grown as annual	2	2	6–8	12–26
Hydrophyllaceae	Spotted nemophila	*Nemophila maculata* Benth.	Annual	4	4	..	C
Boraginaceae	Alkanet	*Anchusa azurea* Mill.	Perennial	8	6	4–20	12–21
	Chinese forget-me-not	*Cynoglossum amabile* Stapf and Drum.	Perennial	5	5	..	C
	True forget-me-not	*Myosotis scorpioides* L.	Perennial grown as annual	4	4	..	C
	Common heliotrope	*Heilotropium peruvianum* L.	Perennial	6	3	..	C

* C indicates that the beet leafhopper completed its life cycle on host plant.

Table 2 continued on page 268.

TABLE 2—(*Continued*)

Family	Name of plant: Common	Name of plant: Scientific	Seasons duration	Number of plants: Inoculated	Number of plants: Virus recovered	Longevity, days: Males	Longevity, days: Females
Labiatae	Scarlet sage	*Salvia splendens* Ker-Gawl.	Perennial grown as annual	2	2	3–8	12–15
Solanaceae	Jasmine tobacco	*Nicotiana alata* Link & Otto var. *grandiflora* Comes	Perennial grown as annual	4	3	6–7	20–30
	Common petunia (Rosy Morn variety)	*Petunia hybrida* Vilm.	Perennial grown as annual	2	2	1–2	1–3
	Scalloped salpiglossis	*Salpiglossis sinuata* Ruiz and Pav.	Annual	4	2	5–8	12–30
	....	*Browallia speciosa* Hook.	Annual	4	4	4–6	6–11
	Wiseton butterflyflower	*Schizanthus wisetonensis* Low.	Annual or biennial	3	3	..	C*
Scrophulariaceae	Yellow foxglove	*Digitalis ambigua* Murr.	Biennial or perennial	7	3	..	C
	Kenilworth ivy	*Cymbalaria muralis* Gaertn., Mey. & Scherb.	Perennial	3	2	3–20	25
	Pouched nemesia	*Nemesia strumosa* Benth.	Annual	2	2	..	C
	Golden monkeyflower	*Mimulus luteus* L.	Perennial	2	2	..	C
Acanthaceae	Black-eyed clockvine	*Thunbergia alata* Bojer	Perennial grown as annual	3	3	3–16	6–51
Cucurbitaceae	Mock-orange gourd	*Cucurbita pepo* L. *ovifera* Bailey	Annual	6	5	3–11	10–49
	Nest-egg gourd	*Cucurbita pepo* L. var. *ovifera* Bailey	Annual	2	2	..	C
	Turks-turban gourd	*Cucurbita pepo* L. var. *ovifera* Bailey	Annual	2	2	7–21	38
	Suakwa towelgourd	*Luffa cylindrica* Roem.	Annual	2	1	..	C
	Calabash gourd	*Lagenaria leucantha* Rusby	Annual	5	3	3–11	3–28
	Dipper-shaped gourd	*Lagenaria leucantha* Rusby	Annual	3	2	4–11	7–26
	Hercules-club gourd	*Lagenaria leucantha* Rusby	Annual	2	2	6–17	24
	Powder-horn gourd	*Lagenaria leucantha* Rusby	Annual	2	2	4–8	8–9
	Spoon gourd	*Lagenaria leuçantha* Rusby	Annual	2	2	9	19
	Snakegourd	*Trichosanthes anguina* L.	Annual	3	3	5–23	13
Lobeliaceae	White-eye lobelia	*Lobelia erinus* L. var. *speciosa*	Annual	10	6	4–14	6–30
	Cardinalflower	*Lobelia cardinalis* L.	Perennial	4	4	8–16	17–26

* C indicates that the beet leafhopper completed its life cycle on host plant.

TABLE 2—(*Concluded*)

Family	Name of plant: Common	Name of plant: Scientific	Seasons duration	Number of plants: Inoculated	Number of plants: Virus recovered	Longevity, days: Males	Longevity, days: Females
Compositae	Crowndaisy	*Chrysanthemum coronarium* L.	Annual	2	2	..	C*
	Feverfew	*Chrysanthemum parthenium* Pers.	Perennial	10	5	..	C
	Marguerite	*Chrysanthemum frutescens* L.	Perennial	2	2	..	C
	Scentless false-camomile	*Matricaria inodora* L.	Annual	6	4	..	C
	Cucumber sunflower	*Helianthus debilis* Nutt.	Annual	7	4	50	..
	Golden thinleaf sunflower	*Helianthus decapetalus* L. var. *multiflorus* Hort.	Perennial	6	6	..	C
	Common zinnia	*Zinnia elegans* Jacq.	Annual	25	25	2–9	..
	Orange zinnia	*Zinnia haageana* Regel	Annual	2	2	3–4	16
	Common cosmos	*Cosmos bipinnatus* Cav.	Annual	2	2	..	C
	Giant-flowering cosmos	*Cosmos bipinnatus* Cav.	Annual	7	4	..	C
	Crested cosmos	*Cosmos hybridus* Hort.	Annual	6	4	..	C
	Calliopsis	*Coreopsis tinctoria* Nutt.	Annual	10	5	..	C
	English daisy	*Bellis perennis* L.	Perennial	3	3	..	C
	Swan-river-daisy	*Brachycome iberidifolia* Benth.	Annual	3	3	..	C
	Bushy arctotis	*Arctotis stoechadifolia* Berg.	Annual	3	3	..	C
	French marigold	*Tagetes patula* L.	Annual	10	6	..	C
	Aztec marigold (African marigold)	*Tagetes erecta* L.	Annual	6	3	..	C
	Winter cape-marigold	*Dimorphotheca aurantiaca* DC.	Perennial	3	3	..	C
	Pot-marigold	*Calendula officinalis* L.	Annual	9	6	..	C
	Rose everlasting	*Helipterum roseum* Benth.	Annual	4	4	..	C
	Strawflower	*Helichrysum bracteatum* Andr.	Annual	6	6	..	C
	Sweet-sultan	*Centaurea moschata* L.	Annual	10	7	..	C
	Basketflower	*Centaurea americana* Nutt.	Annual	5	3	..	C
	Cornflower	*Centaurea cyanus* L.	Annual	6	6	..	C

* C indicates that the beet leafhopper completed its life cycle on host plant.

HOST RANGE OF CURLY TOP AMONG ORNAMENTAL FLOWERING PLANTS

The number of ornamental flowering plants inoculated with curly top and the number of infected plants from which the virus was recovered by previously noninfective beet leafhoppers and transferred to beet seedlings is shown in table 2. The host range of curly top among ornamental flowering plants includes 92 species in 73 genera belonging to 33 families.

Many species of ornamental flowering plants repeatedly inoculated by different lots of infective beet leafhoppers showed no foliage symptoms and the virus was not recovered from such plants. These plants apparently are either immune or resistant to curly top and are not listed in table 2.

SYMPTOMS OF CURLY TOP

Environmental conditions are important factors influencing the expression of curly-top symptoms. High humidity and high temperatures with a range of 70° to 110° F were maintained in the greenhouse during the summer months. Certain species of plants which displayed no outstanding symptoms of curly top might have developed them under different humidity and temperatures.

Ornamental flowering plants infected with curly top show a variation in symptoms. Many plants develop some of the symptoms developed by the sugar beet as described in a previous paper.[22] Other infected plants such as Madagascar periwinkle (*Vinca rosea*), common heliotrope (*Heliotropium peruvianum*), and purple candytuft (*Iberis umbellata*) did not develop symptoms. Either such species are symptomless carriers of the disease or the symptoms are entirely masked under greenhouse conditions.

The effect of the disease on the flowers of many species was not studied in detail. Young plants infected with curly top frequently produce no flowers as they mature. Older plants infected before blooming often develop dwarfed, malformed, and fewer flowers than healthy plants. Some species of infected plants produce normal flowers.

A brief description of symptoms on the individual species follows. These symptoms are described in many instances from young infected plants. Such symptoms are often more pronounced than those shown by plants infected at a later stage of development. Plants grown from seeds in the greenhouse and enclosed in cages often assume a spindling habit, and the symptoms of curly top on such plants are often different from those shown by plants naturally infected in the field.

Moraceae, Mulberry Family

On Japanese hop (*Humulus japonicus*) infected with curly top no symptoms were apparent.

Chenopodiaceae, Goosefoot or Saltbush Family

Common summer cypress (*Kochia trichophylla*) infected with curly top showed severe foliage symptoms and developed secondary shoots from the axils of the leaves. The terminal leaves of the branches and axillary shoots were dwarfed and twisted, sometimes in a spiral. The margin and midrib of the leaves were sinuous, with knot-like swellings on the latter. The leaves showed blister-like elevations on the lower surface and cleared veinlets.

Amaranthaceae, Amaranth Family

Amaranthus aurora infected with curly top was stunted, having shortened internodes and secondary shoots arising from the axils of the leaves. The terminal leaves of the main and secondary shoots were dwarfed, with the outer margin rolled inward, and with the petioles often bent downward or twisted (plate 1, *A*). The lateral veins were sinuous, and developed knot-like swellings, and the veinlets were transparent.

On Josephs-coat (*Amaranthus gangeticus*) the symptoms of curly top were somewhat similar to those described on *A. aurora*. The leaf tissue between the protruding lateral veins was sunken in such a manner that the leaf resembled a corkscrew (plate 1, *B*).

On love-lies-bleeding (*Amaranthus caudatus*) the symptoms of curly top were similar to those described on *A. aurora*. Infected plants kept out of doors developed a compact mass of dwarfed, curled leaves at the apexes of the stems.

Feather cockscomb (*Celosia argentea*) infected with curly top was stunted and developed numerous axillary shoots. The pluming heads were dwarfed and had few branches (fig. 1), which were dark red or brownish red in color instead of the normal scarlet.

On common globe-amaranth (*Gomphrena globosa*) the symptoms of curly top were similar to those described on *Amaranthus aurora*.

Nyctaginaceae, Four-o'Clock Family

Common four-o'clock (*Mirabilis jalapa*) infected with curly top was stunted and developed dark, dull green leaves on the axillary shoots. The youngest leaves were cupped outward and the veinlets were transparent.

PORTULACACEAE, PURSLANE FAMILY

Common portulaca (*Portulaca grandiflora*) infected with curly top was stunted and chlorotic, bearing numerous axillary dwarfed leaves.

Common rockpurslane (*Calandrinia grandiflora*) infected with curly top was stunted, the older leaves cupped outward, and the younger dwarfed leaves formed a compact mass of curled leaves (fig. 2).

Fig. 1.—Feather cockscomb (*Celosia argentea*): left, conical pluming head from healthy plant; right, dwarfed pluming heads from two plants infected with curly top, showing reduction in number and size of branches.

CARYOPHYLLACEAE, PINK FAMILY

Sweet william (*Dianthus barbatus*) infected with curly top was stunted, and had shortened internodes. The younger leaves at the apexes of the shoots were dwarfed, yellow, and curled, while the somewhat older leaves were often twisted (fig. 3) and had sinuous veins, cleared veinlets, and protuberances on the lower surface.

Fig. 2.—Common rockpurslane (*Calandrinia grandiflora*) infected with curly top, showing stunted plant with the older leaves cupped outward and the younger leaves forming a compact mass of curled leaves.

Fig. 3.—Sweet william (*Dianthus barbatus*): left, tip of a shoot from a healthy plant; right, apical end of a shoot from a plant infected with curly top, showing dwarfed, curled, younger leaves, and twisted older leaves.

Chinese pink (*Dianthus chinensis*) infected with curly top was stunted and developed secondary shoots from the axils of the leaves (fig. 4, *A*). The older leaves were curled downward (fig. 5, *A*), sometimes in a circle or spiral (fig. 4, *A*); they became chlorotic in the later stages of the

Fig. 4.—*A*, Chinese pink (*Dianthus chinensis*): left, plant experimentally infected with curly top by infective beet leafhoppers, showing axillary shoots with dwarfed and twisted leaves. The older leaves are curled downward, sometimes in a circle or spiral. Right, healthy check or control plant on which noninfective leafhoppers had fed. *B*, Spiderflower (*Cleome spinosa*) infected with curly top, showing secondary shoots forming a cluster near the apical end of the plant owing to shortened internodes. The leaves at the crown of the plant are dwarfed and cupped inward. The petioles of the older leaves are bent down.

disease, and usually had deep green veins. The leaves on the axillary shoots were dwarfed and twisted (fig. 4, *A*). Protuberances were present on the lower surface of the leaves.

Heddewig pink (*Dianthus chinensis* var. *heddewigii*) infected with curly top was stunted, with shortened internodes, and numerous axillary shoots. The leaves were curled downward and were often twisted. Protuberances resembling tiny warts were present on the lower surface of the leaves with pits or depressions on the upper surface of the leaves corresponding to the position of the small elevations on the under side.

On grass pink (*Dianthus plumarius*) the symptoms of curly top were similar to those described for Chinese pink (*D. chinensis*).

Carnation (*Dianthus caryophyllus*) developed leaves at the apex of infected plants and on the secondary shoots which were chlorotic and curled downward.

Babysbreath (*Gypsophila paniculata*) infected with curly top showed no symptom of the disease under greenhouse conditions.

Fig. 5.—*A*, Chinese pink (*Dianthus chinensis*) infected with curly top, showing stunted plant with older leaves curled downward and with dwarfed, filamentous, terminal leaves. *B*, Common mignonette (*Reseda odorata*): left, floral inflorescence from a plant infected with curly top, showing flowers with sepals and no corollas; right, stem from an infected plant showing axillary shoots.

Drooping catchfly (*Silene pendula*) infected with curly top developed young leaves which were dwarfed, twisted, sometimes folded inward along the midrib, and had knot-like swellings on the sinuous veins.

Maltese cross (*Lychnis chalcedonica*) infected with curly top developed numerous secondary shoots with dwarfed terminal leaves showing an inward curl or rolling of the outer margin. The veins were distorted with knot-like swellings, and wart-like protuberances occurred on the lower surface of the leaves. The veinlets on the youngest leaves were cleared.

On Haage campion (*Lychnis haageana*) the symptoms of curly top were similar to those described on Maltese cross.

Ranunculaceae, Crowfoot Family

Poppy anemone (*Anemone coronaria*) infected with curly top was stunted and chlorotic. The leaves were cupped downward (fig. 6) with petioles often twisted.

Love-in-a-mist (*Nigella damascena*) infected with curly top showed a marked stunting of the crown and developed dwarfed, yellow leaves, while the petioles of the older leaves were often curled downward (fig. 7).

Fig. 6.—Poppy anemone (*Anemone coronaria*): upper row, three leaves from a plant experimentally infected with curly top, showing downward-cupped leaves; lower row, two leaves from a healthy plant.

Older infected plants developed axillary shoots, which again developed secondary shoots forming a dense cluster. In the later stage of the disease, the plants were chlorotic.

Orange larkspur (*Delphinium nudicaule*) infected with curly top failed to develop symptoms of the disease under greenhouse conditions.

Papaveraceae, Poppy Family

Iceland poppy (*Papaver nudicaule*) infected with curly top had the youngest leaves dwarfed and either cupped inward or outward (plate 2, *B*). A yellow discoloration developed between the veins of the older leaves, while the area in the vicinity of the veins retained the green color for a long time (plate 2, *B*).

The varieties of Oriental poppy (*Papaver orientale*) infected with curly top included an unnamed variety, the horticultural variety

Fig. 7.—Love-in-a-mist (*Nigella damascena*): upper, three leaves from a plant infected with curly top, showing crooked petioles; lower, terminal shoot, showing marked stunting of the crown and dwarfed leaves.

Salmon Queen, and an Oriental poppy hybrid. All were stunted and chlorotic and had leaves cupped inward along the midrib.

Capparidaceae, Caper Family

Spiderflower (*Cleome spinosa*) infected with curly top was stunted, with shortened internodes, and developed secondary shoots from the axils of the leaves which formed a cluster near the apical end of the

Fig. 8.—Spiderflower (*Cleome spinosa*): upper, two leaves from a healthy plant; lower, six leaves from a plant infected with curly top showing discoloration, from mottling to yellowing.

plant (fig. 4, *B*). The petioles of the older leaves often drooped (fig. 4, *B*). The leaves on the axillary shoots and on the apical end of the plant were dwarfed, curled inward (fig. 4, *B* and plate 1, *C*), and the tissue between the protruding lateral veins was sunken. The veinlets were transparent. In the later stages of the disease the leaves became mottled and chlorotic (fig. 8).

Cruciferae, Mustard Family

On annual stock (*Matthiola incana* var. *annua*) the most conspicuous symptom of curly top was the light-brown exudations from the petioles and leaves, which later became dark brown in color. The plants were stunted and had shortened internodes and numerous axillary shoots bearing dwarfed leaves at the apical end of the plant. The lower leaves were dry and the upper older leaves thick and leathery. Young plants infected with curly top failed to develop flowers as they matured.

Dames rocket (*Hesperis matronalis*), infected with curly top, was stunted and had numerous secondary shoots arising from the axils of the leaves. The younger leaves and those of the axillary shoots were dwarfed and formed a compact mass, from which projected the older, apparently normal leaves. The veinlets of the younger leaves were transparent.

Honesty (*Lunaria annua*) infected with curly top showed no symptoms of the disease under greenhouse conditions.

Purple candytuft (*Iberis umbellata*) infected with curly top showed no symptoms of the disease under greenhouse conditions.

Resedaceae, Mignonette Family

Common mignonette (*Reseda odorata*) infected with curly top developed numerous secondary shoots near the growing tips of the stems (fig. 5, *B*, p. 275), bearing dwarfed leaves curled downward, with sinuous margins and distorted veins. Protuberances were present on the lower surface of the older leaves. The inflorescence showed flowers with sepals, but no corollas (fig. 5, *B*) or with withered corollas.

Geraniaceae, Geranium Family

Fish geranium (*Pelargonium hortorum*), grown from cuttings and infected with curly top, was stunted and developed an inward rolling of the basal margin and an inward or outward cupping of the leaves. The youngest leaves showed cleared veinlets and protuberances on the lower surface. In the later stages of the disease, the lower leaves became chlorotic. Single giant-flowering hybrid geraniums grown from seeds infected with curly top developed symptoms similar to those on fish geranium.

Tropaeolaceae, Tropaeolum Family

Canary nasturtium (*Tropaeolum peregrinum*) infected with curly top, showed axillary shoots, twisted petioles, and dwarfed curled younger leaves (fig. 9). Near the terminal ends of the branches, the lobes of the older leaves were often curled.

Euphorbiaceae, Spurge Family

Snow-on-the-mountain (*Euphorbia marginata*) infected with curly top showed cleared veinlets on the younger, dwarfed, inward-cupped leaves (fig. 10). The older leaves were cupped outward and drooped from the stem, and in the later stages of the disease turned yellow.

Fig. 9.—Canary nasturtium (*Tropaeolum peregrinum*): left, apical shoot from a plant infected with curly top, showing small secondary shoots arising from the axils of the leaves; dwarfed, curled younger leaves; curled lobes of older leaves; and twisted petioles. Right, healthy check or control plant on which noninfective beet leafhoppers had fed.

Fig. 10.—Snow-on-the-mountain (*Euphorbia marginata*): left, apical end of a branch from a healthy plant; right, apical end of a branch from a plant infected with curly top, showing cleared veinlets on the younger dwarfed, inward-cupped leaves. The older leaves are cupped outward and the lower leaves droop from the stem.

Sapindaceae, Soapberry Family

Balloonvine (*Cardiospermum halicacabum*) infected with curly top was stunted and developed secondary shoots from the axils of the leaves. Infected plants showed severe foliage symptoms. The leaves of the terminal,

Fig. 11.—Balloonvine (*Cardiospermum halicacabum*): branch from a plant infected with curly top, showing severe foliage symptoms. The leaves of the terminal, lateral, and axillary shoots are dwarfed, curled, and twisted in clumps. The margins of the older leaves are rolled toward the midrib, with the tissue sunken between the lateral veins resulting in protruding veins.

lateral, and axillary shoots were dwarfed, curled, and twisted in clumps (fig. 11). The margin of the older leaves was rolled toward the midrib, and the tissue was sunken between the lateral veins, which resulted in protruding distorted veins (fig. 11). Wart-like protuberances were present on the lower surface of the leaves.

Malvaceae, Mallow Family

Herb treemallow (*Lavatera trimestris*) infected with curly top was stunted, and developed outward-cupped leaves showing transparent venation. The plants were very susceptible to the disease and wilted and died prematurely.

Violaceae, Violet Family

Tufted pansy (*Viola cornuta*) infected with curly top was stunted, had shortened internodes, and developed secondary shoots from the axils of the leaves. The leaves of the axillary shoots were dwarfed and sometimes cupped along the midrib, or the tips of the leaves were rolled toward the midrib.

Onagraceae, Evening-Primrose Family

Clarkia (*Clarkia elegans*) infected with curly top was stunted and had shortened internodes. The youngest leaves were dwarfed, sometimes twisted, or with the outer margin rolled inward. The older leaves often drooped.

Umbelliferae, Parsley Family

Blue laceflower (*Trachymene caerulea*) infected with curly top was stunted and chlorotic. The leaves at the apical end of the plant were cupped outward and sometimes the cupping continued until each leaf resembled a small ball. The petioles were bent downward and became dry (plate 4, *B*) and often the entire plant wilted and died prematurely. The flowers were dwarfed and malformed (plate 4, *C*).

Primulaceae, Primrose Family

Top primrose (*Primula obconica*) infected with curly top was stunted. The youngest leaves were dwarfed, cupped outward, and chlorotic between the veins, while the area in the vicinity of the veins remained green. The veinlets were transparent.

Primula saxatalis infected with curly top was stunted, and the youngest leaves were cupped outward (fig. 12, *A*).

Cowslip primrose (*Primula veris*) infected with curly top was stunted and chlorotic.

Oxlip primrose (*Primula elatior*) infected with curly top was stunted and chlorotic, often wilted, and died prematurely.

Plumbaginaceae, Plumbago or Leadwort Family

On notchleaf sea-lavender (*Limonium sinuatum*), the youngest leaves of infected plants were dwarfed and chlorotic.

Fig. 12.—*A*, *Primula saxatalis:* leaves from a plant infected with curly top, showing outward-cupped leaves. *B*, Nest-egg gourd (*Cucurbita pepo* var. *ovifera*): axillary shoots from an infected plant, showing dwarfed, puckered, balled leaves, with shortened petioles, and with dwarfed flowers. *C*, Spoon gourd (*Lagonaria leucantha*): terminal shoot from an infected plant, showing outward-cupped leaves, and dwarfed tendrils.

Apocynaceae, Dogbane Family

Madagascar periwinkle (*Vinca rosea*) infected with curly top showed no symptoms under greenhouse conditions.

Convolvulaceae, Morning-Glory Family

Crimson starglory (*Quamoclit lobata*) infected with curly top showed an inward rolling of the margin of the older leaves with wart-like pro-

tuberances on the lower surface (plate 2, *C*). The younger leaves were twisted and developed bent petioles.

Brazilian morning-glory (*Ipomoea setosa*) infected with curly top showed an inward rolling of the margin of the older leaves, puckering of the blades, transparent venation (plate 3, *A*), and sometimes wart-like protuberances on the lower surface of the leaves. The younger leaves were sometimes folded inward along the midrib (plate 3, *A*).

Fig. 13.—Drummond phlox (*Phlox drummondii*) infected with curly top, showing stunted plant, with shortened internodes, and dwarfed, curled leaves.

Polemoniaceae, Phlox Family

Drummond phlox (*Phlox drummondii*) infected with curly top was stunted, with shortened internodes (fig. 13), and had secondary shoots arising from the axils of the leaves. The leaves on the axillary shoots were dwarfed, linear near the tips, cupped inward, and chlorotic. The midrib of the older leaves was sinuous and the veinlets were transparent. The flowers near the apexes of the secondary shoots developed sepals but no petals.

On purplebell cobaea (*Cobaea scandens*) infected with curly top the apical leaflets were dwarfed and yellow, their protruding lateral veins resembled a corkscrew, and the petioles bent downward. The somewhat older leaves showed clear veinlets.

Hydrophyllaceae, Water-leaf Family

Spotted nemophila (*Nemophila maculata*) infected with curly top failed to show symptoms of the disease under greenhouse conditions.

BORAGINACEAE, BORAGE FAMILY

Alkanet (*Anchusa azurea*) infected with curly top was stunted. Its youngest leaves were dwarfed.

Chinese forget-me-not (*Cynoglossum amabile*) infected with curly top was stunted, had shortened internodes, and developed axillary shoots. The youngest leaves were dwarfed, curled, and twisted (fig. 14).

Fig. 14.—Chinese forget-me-not (*Cynoglossum amabile*): left, terminal and lateral shoots from a healthy plant; upper right, flower stalks forming a dense cluster at the terminal end of a branch from an infected plant; lower right, lateral shoot, showing dwarfed, curled, and twisted leaves.

Brown droplets exuded from the leaves and stem. The flower stalks from numerous axillary shoots formed a dense cluster (fig. 14) and the flowers were frequently dwarfed and reduced in number.

True forget-me-not (*Myosotis scorpioides*) infected with curly top was stunted, and developed numerous secondary shoots. The youngest leaves were dwarfed, almost linear in shape near the apexes of the shoots, and chlorotic. The older leaves were curled downward with the

margin cupped inward. The leaves on the secondary shoots were dwarfed and had a sinuous midrib and protuberances on the veins.

Common heliotrope (*Heliotropium peruvianum*) infected with curly top failed to show symptoms of the disease under greenhouse conditions.

Labiatae, Mint Family

Scarlet sage (*Salvia splendens*) infected with curly top and kept out of doors was stunted, had shortened internodes, and developed a compact mass of axillary shoots. The margins of the leaves were often rolled in-

Fig. 15.—Scalloped salpiglossis (*Salpiglossis sinuata*) infected with curly top, showing secondary shoots arising from the axils of the leaves and base of the plant. The leaves of the apical end of the plant and on the axillary shoots are rolled or curled inward. The older leaves are cupped outward.

ward (plate 3, *C*), or cupped inward along the midrib, the veinlets were transparent, and the petioles were bent downward. The leaves near the tips of the secondary shoots were dwarfed and chlorotic. The flowers were reduced in number, dwarfed, often failed to expand, and some remained green instead of the normal brilliant scarlet color.

Solanaceae, Nightshade Family

Jasmine tobacco (*Nicotiana alata* var. *grandiflora*) infected with curly top was stunted and had shortened internodes. The youngest leaves were dwarfed, cupped outward, and had cleared veinlets.

Common petunia (*Petunia hybrida*) (Rosy Morn variety) infected with curly top was stunted and developed numerous secondary shoots bearing dwarfed leaves. Protuberances were present on the veins on the lower surface of the leaves of the secondary and apical shoots and gave the veins a roughened appearance. The corolla of the flowers often failed to expand and became dry. In the later stages of the disease the entire plant turned yellow and died.

Scalloped salpiglossis (*Salpiglossis sinuata*) infected with curly top was stunted and had secondary shoots arising from the axils of the leaves

Fig. 16.—Wiseton butterflyflower (*Schizanthus wisetonensis*): shoots and leaves from a plant infected with curly top, showing semicircular and circular curling of the petioles of the younger leaves.

and base of the plant (fig. 15). The dwarfed, chlorotic leaves on the terminal end of the plant and on the axillary shoots were rolled or curled inward. The older leaves were cupped outward. The veinlets were transparent (plate 3, *B*) and wart-like protuberances were present on the lower surface of the leaves.

On *Browallia speciosa* the terminal leaflets of infected plants were dwarfed, cupped inward along the midrib, and had protruding lateral veins. The veinlets were transparent at the base of the older leaves, and the petioles were bent downward.

Wiseton butterflyflower, *Schizanthus wisetonensis* (*S. pinnatus* × *S. grahami*) infected with curly top showed a circular or semicircular curling of the petioles of the younger leaves (fig. 16). The leaves on the secondary shoots arising from the axils of the leaves were dwarfed and had curled petioles. Infected plants often wilted and died prematurely.

Scrophulariaceae, Figwort Family

Yellow foxglove (*Digitalis ambigua*) infected with curly top developed youngest leaves which were dwarfed, cupped inward along the midrib, and the veinlets were transparent.

Kenilworth ivy (*Cymbalaria muralis*) infected with curly top developed a large number of dwarfed leaves on the secondary shoots at the

Fig. 17.—Kenilworth ivy (*Cymbalaria muralis*): apical end of branch from a plant infected with curly top, showing dwarfed leaves on the secondary shoots at the nodes and bent and looped petioles.

nodes (fig. 17) instead of rooting at the nodes. The larger leaves at the apexes of the branches were slightly cupped inward with wart-like protuberances on the lower surface of the leaves and with bent or looped petioles (fig. 17).

Pouched nemesia (*Nemesia strumosa*) infected with curly top was stunted and chlorotic. The veinlets were transparent on the youngest leaves.

Golden monkeyflower (*Mimulus luteus*) infected with curly top failed to show symptoms of the disease under greenhouse conditions.

Acanthaceae, Acanthus Family

Black-eyed clockvine (*Thunbergia alata*) infected with curly top developed secondary shoots with dwarfed, puckered, yellow leaves (plate 2, *A*). The veinlets were transparent (plate 4, *E*). The petioles were often bent downward.

CUCURBITACEAE, GOURD FAMILY

The ten ornamental gourds listed in table 2 were experimentally infected with curly top. The symptoms of curly top were the same on all ten. The plants were stunted and developed secondary shoots bearing chlorotic, dwarfed, puckered, outward-cupped leaves with shortened petioles (fig. 12, *B*, p. 283). The leaves on the secondary shoots resembled a small ball.

Fig. 18.—Cardinalflower (*Lobelia cardinalis*): left, healthy plant; right, plant infected with curly top, showing dwarfed, inward-curled young leaves and malformed, twisted, older leaves.

The older leaves were cupped outward (fig. 12, *C*), and dull green. The tendrills usually were dwarfed. The veinlets were transparent. The flowers in the axils of the leaves were sessile, and their corollas failed to expand and often withered prematurely.

LOBELIACEAE, LOBELIA FAMILY

White-eye lobelia (*Lobelia erinus* var. *speciosa*) infected with curly top was stunted, and had numerous secondary shoots bearing dwarfed, chlorotic leaves.

Cardinalflower (*Lobelia cardinalis*) infected with curly top was stunted. The youngest leaves were dwarfed, puckered, with inward-curled margins and cleared veinlets. The oldest leaves were malformed and twisted (fig. 18).

Compositae, Composite Family

Crowndaisy (*Chrysanthemum coronarium*) infected with curly top was stunted, chlorotic, and had dwarfed, curled leaves.

Feverfew (*Chrysanthemum parthenium*) infected with curly top failed to show symptoms of the disease under greenhouse conditions.

Marguerite (*Chrysanthemum frutescens*) infected with curly top developed a dense cluster of dwarfed, chlorotic leaves at the apexes of the stems and secondary shoots. The leaflets were curled inward, outward, or folded inward along the midrib with bent petioles (plate 4, *D*).

Scentless false-camomile (*Matricaria inodora*) infected with curly top was stunted, chlorotic, and developed numerous secondary shoots arising from the axils of the leaves. The youngest leaves were dwarfed, curled, and twisted.

Cucumber sunflower (*Helianthus debilis*) infected with curly top developed chlorotic, secondary shoots from the axils of the leaves. The flowers were dwarfed (plate 4, *A*).

Golden thinleaf sunflower (*Helianthus decapetalus* var. *multiflorus*), infected with curly top failed to show symptoms under greenhouse conditions.

All varieties of common zinnia (*Zinnia elegans*) grown in California were experimentally infected with curly top. Infected plants were stunted, and had inward-cupped leaves showing cleared veinlets.

On orange zinnia (*Zinnia haageana*) the symptoms were similar to those described on common zinnia.

The cosmos varieties and species infected with curly top were common cosmos (*Cosmos bipinnatus*), giant-flowering cosmos (*Cosmos bipinnatus*) and crested cosmos (*Cosmos hybridus*). The symptoms on the three were similar. Infected plants were stunted, chlorotic, and had shortened internodes and secondary shoots arising from the axils of the leaves. The leaflets were curled and twisted with the petioles sometimes bent downward.

Calliopsis (*Coreopsis tinctoria*) infected with curly top developed secondary shoots with circular twisted leaves. The leaves on other portions of the plants showed no symptoms of the disease except drooping of the petioles. The terminal flower heads on the secondary shoots were dwarfed.

On English daisy (*Bellis perennis*) the youngest leaves infected with curly top were dwarfed, malformed, and cupped inward. The veinlets were transparent, and wart-like protuberances were present on the lower surface of the leaves.

Swan-river-daisy (*Brachycome iberidifolia*) infected with curly top developed secondary shoots from the axils of the leaves terminating in tufts of dwarfed, twisted, yellow leaves (fig. 19). A tangled mass of leaves was present at the apical end of the plant and at the end of the stunted branches. The petioles of the older leaves were often curled downward, upward, or twisted.

Bushy arctotis (*Arctotis stoechadifolia*) infected with curly top de-

Fig. 19.—Swan-river-daisy (*Brachycome iberidifolia*): branch from a plant experimentally infected with curly top, showing secondary shoots arising from the axils of the leaves and terminating in a tuft of dwarfed, twisted leaves.

veloped leaves on the secondary shoots which were dwarfed and had the lobes curled inward.

French marigold (*Tagetes patula*) infected with curly top developed axillary shoots bearing dwarfed leaves with the petioles curled downward. The flower buds were yellow instead of green and the peduncles were often curled downward.

Aztec marigold, or African marigold (*Tagetes erecta*) infected with curly top failed to show symptoms under greenhouse conditions.

Winter cape-marigold (*Dimorphotheca aurantiaca*) infected with curly top was stunted, and developed shortened internodes and axillary shoots. In the advanced stage of the disease the terminal leaves were dwarfed, curled, and yellow (fig. 20). The teeth-like projections of the leaves were often curled inward. The longitudinal veins on the lower surface of the leaves were distorted.

Pot-marigold (*Calendula officinalis*) infected with curly top failed to develop symptoms under greenhouse conditions.

Rose everlasting (*Helipterum roseum*) infected with curly top developed terminal shoots which were either erect or drooping with a cluster

Fig. 20.—Winter cape-marigold (*Dimorphotheca aurantiaca*): branch from a plant infected with curly top, showing dwarfed, curled leaves at the tip of the stem and also on the secondary shoots arising from the axils of the leaves. The teeth-like projections of the leaves are often curled inward.

of twisted yellow leaves around the bud (fig. 21). The margin of the leaves below the bud were sinuous.

When small plants of strawflower (*Helichrysum bracteatum*) were infected with curly top they were stunted, their youngest leaves were dwarfed, twisted, and yellow in the advanced stage of the disease. The somewhat older leaves were also twisted and the veinlets were transparent. Plants infected at a later stage of development had numerous secondary shoots toward the tip of the branches with twisted leaves showing knot-like swellings on the veins.

Sweet-sultan (*Centaurea moschata*) infected with curly top failed to develop symptoms of curly top under greenhouse conditions.

Basketflower (*Centaurea americana*) infected with curly top failed to show symptoms of the disease under greenhouse conditions.

On cornflower (*Centaurea cyanus*) infected with curly top, the leaves of the secondary shoots were dwarfed and sometimes two adjacent leaves were coiled downward.

Fig. 21.—Rose everlasting (*Helipterum roseum*): tips from plant infected with curly top, showing erect or drooping terminal shoots with clusters of twisted leaves around the buds.

LONGEVITY AND LIFE CYCLE OF THE BEET LEAFHOPPER

A comparative record was obtained of the longevity of male and female beet leafhoppers on plants on which the insects were not able to complete their life history. Adults of the spring or summer generations were used and not the dark adults of the overwintering generation. Two plants of each variety or species were inoculated with curly top by either male or female leafhoppers, and, by daily examination of each cage, a record was obtained of the longevity of the last living male and female as indicated in table 2. When the insects died after a short exposure on the host plant, presumably owing to unfavorable food, the plants were repeatedly inoculated by different lots of insects and the range in the longevity was determined. The longevity of the adults was often shorter on young plants than on older ones.

A record was kept of all host plants on which the nymphs, after hatching from the egg, completed their nymphal stages and acquired the winged stage. The host plants of curly top on which the beet leafhopper completes its life cycle are indicated in table 2. There was considerable variation in the percentage of specimens reared to the adult stage on the different host plants. This difference in population was probably due to the variation in the amount of suitable food provided by the plant.

On unfavorable food plants, the nymphs hatched but soon died. The nymphal stages were often prolonged, presumably owing to unfavorable food.

The plants which were proved susceptible to curly top were not always favorable food plants of the leafhopper. Plants which were suitable food plants and on which the insects were able to live for long periods were often found resistant or immune to curly top. On the other hand, the leafhoppers often transmitted curly top to certain plants that were unfavorable food plants, and on which they could live for only a few days.

SUMMARY

Curly top was experimentally transmitted to 92 species of ornamental flowering plants in 73 genera belonging to 33 families. The virus was recovered from each species or variety of infected plant by previously noninfective beet leafhoppers and transferred to sugar beets. The host plants of curly top among ornamental flowering plants include 47 species of annuals, 2 species of annuals or biennials, 11 species of perennials grown as annuals, 3 species of biennials or perennials, and 30 species of perennials.

Ornamental flowering plants infected with curly top show a variation in symptoms. Fifteen species, including 6 species of annuals, 2 species of annuals grown as perennials, and 7 species of perennials, failed to develop symptoms of the disease under greenhouse conditions.

Many of the plants infected with curly top were stunted and developed numerous secondary shoots from the axils of the leaves. Chlorosis and a development of shortened internodes was often characteristic of infected plants.

The leaves of some infected plants were dwarfed and often developed an inward rolling of the outer margin, and later the entire blade showed a pronounced inward curling toward the midrib. Other species showed an outward rolling of the leaves toward the midrib. In still other species the leaves may be cupped inward or outward, or they may be twisted, sometimes in a spiral. Puckering and mottling of the leaves occur in certain species and malformation of the leaves in others.

Many infected ornamental flowering plants developed cleared veins, a reliable symptom of curly top on the sugar beet. Another reliable symptom of curly top is the roughened appearance on the lower surface of the leaves developing after the veinlets have cleared. The veins develop numerous small elevations resembling tiny warts. As the disease progresses, nipple-like papillae and knot-like swelling resembling galls develop here and there on the distorted, thickened veins.

Some infected plants, such as annual stock (*Matthiola incana* var. *annua*) and Chinese forget-me-not (*Cynoglossum amabile*) exude droplets of brown liquid from the veins, midrib, petioles, and stems.

Wilting and premature death of the plants occur with blue laceflower (*Trachymene caerulea*), Wiseton butterflyflower (*Schizanthus wisetonensis*), and herb treemallow (*Lavatera trimestris*).

Young plants infected with curly top frequently produce no flowers as they mature. Older plants infected before blooming often develop dwarfed, malformed, and fewer flowers than healthy plants.

The life cycle of the beet leafhopper was completed on 65 species of ornamental flowering plants in 51 genera, belonging to 23 families.

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Plate 1.—*A*, Terminal shoot of *Amaranthus aurora* infected with curly top, showing dwarfed, terminal leaves with the outer margin rolled inward and with the petioles bent downward or twisted. *B*, Terminal shoot of Josephs-coat (*Amaranthus gangeticus*) with the tissue sunken between the lateral veins and with protuding veins resembling a corkscrew. *C*, Terminal shoot of spiderflower (*Cleome spinosa*) infected with curly top, showing dwarfed, inward-curled leaflets.

Plate 2.—*A*, Black-eyed clockvine (*Thunbergia alata*): chlorotic leaf from a plant infected with curly top. *B*, Iceland poppy (*Papaver nudicaule*): left, two small leaves from an infected plant showing outward and inward cupping; right, three leaves showing successive stages of yellowing between the veins, while the area along the veins remained green for a long time. *C*, Crimson starglory (*Quamoclit lobata*): left, large leaf from a healthy plant used as a check or control on which noninfective beet leafhoppers had fed; right, large leaf from a plant infected with curly top showing protuberances on the lower surface; small leaves showing malformations.

Plate 3.—*A*, Brazilian morning-glory (*Ipomoea setosa*): right and left leaves, from a plant infected with curly top showing inward rolling of the margin and transparent venation; lower center, leaf folded inward along the midrib; upper center, leaf from a healthy plant used as a check or control on which noninfective beet leafhoppers had fed. *B*, Scalloped salpiglossis (*Salpiglossis sinuata*): leaf showing cleared veinlets. *C*, Scarlet sage (*Salvia splendens*): apical shoot from a plant infected with curly top showing inward roll of the margin of the youngest leaves, cleared veinlets, and bent petioles.

Plate 4.—*A*, Cucumber sunflower (*Helianthus debilis*): upper, flower from a healthy plant; lower, dwarfed flower from a plant infected with curly top. *B*, Blue laceflower (*Trachymene caerulea*): left, healthy plant which had been exposed to noninfective beet leafhoppers; right, stunted plant infected with curly top by infective beet leafhoppers showing outward-cupped leaves and dried petioles. *C*, Blue laceflower (*Trachymene caerulea*): left, normal flower from a healthy plant; right, dwarfed and malformed flower from an infected plant. *D*, Marguerite (*Chrysanthemum frutescens*): secondary shoots and leaves from a plant infected with curly top, showing curled leaflets. *E*, Black-eyed clockvine (*Thunbergia alata*): leaf from a plant infected with curly top showing cleared veinlets.

NEGATIVE EVIDENCE ON MULTIPLICATION OF CURLY-TOP VIRUS IN THE BEET LEAFHOPPER, EUTETTIX TENELLUS

JULIUS H. FREITAG

NEGATIVE EVIDENCE ON MULTIPLICATION OF CURLY-TOP VIRUS IN THE BEET LEAFHOPPER, EUTETTIX TENELLUS[1, 2, 3]

JULIUS H. FREITAG[4]

INTRODUCTION

MULTIPLICATION of certain viruses in their insect vectors and the consequent theory of biological relation between insects and viruses have been generally surmised on the basis of insufficient evidence. For many virus diseases a definite interval of time has been reported as necessary after an insect has fed on a diseased host before it is able to transmit a virus to a healthy host. This interval has been called the "incubation period" of the virus in the insect. Some writers have suggested that this may be a period during which the virus undergoes some developmental phase in a possible life cycle in the body of the insect. Insect vectors of virus diseases have also frequently been reported to remain infective during their entire adult life, after once having acquired the virus by feeding on a diseased host.

Multiplication of Viruses in Insects.—Few experiments have been conducted which give any definite evidence on the question of the multiplication of viruses in their insect vectors. Davis, Frobisher, and Lloyd,[(7)][5] working with yellow fever and the mosquito, *Aedes aegypti* L., have demonstrated that the quantity of virus present in the vector never increases beyond that found immediately after an infective meal. The quantity of virus was determined by finding the greatest dilution of the bodies of crushed mosquitoes that would give infection when inoculated into healthy animals. During the two weeks after a meal of infectious blood there occurred a reduction in the quantity of virus to approximately 1 per cent of that in recently fed insects. There was an increase in titratable virus at a later period, but they suggest that this rise signi-

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[2] Presented as a thesis to the graduate division of the University of California, May, 1935, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[3] It is a pleasure to express gratitude to Dr. H. H. P. Severin, who suggested the problem and under whose supervision this investigation was conducted.

[4] Junior Entomologist in the Experiment Station.

[5] Superscript numbers in parentheses refer to "Literature Cited" at the end of the paper.

fies not a multiplication, but merely an increase in extracellular virus. At no later period did the quantity of virus equal that demonstrable in freshly fed insects, and the authors, therefore, conclude that no multiplication of the virus occurs in the yellow-fever mosquito.[6]

Fukushi[10] has obtained evidence which indicates that a multiplication of the rice-dwarf virus occurs in the insect vector *Nephotettix apicalis* var. *cincticeps*. He demonstrated that the virus was transmitted through the eggs of the infective leafhoppers to the progeny of the third generation. Apparently the only explanation for the retention of the rice-dwarf virus by the leafhopper for three generations without having access to a source of infection is that the virus must have multiplied in the vector. This transmission of rice-dwarf virus through the egg of the infective leafhopper to its progeny is the only record of such an occurrence in plant-virus literature.

Mechanical Transmission of Plant Viruses by Insects.—There are several examples of the transmission of the plant viruses by insects in such brief periods as to preclude multiplication of the virus in the vector and to indicate that in these cases at least the transmission must have been purely mechanical. Doolittle and Walker[8] have shown that the striped cucumber beetle, *Diabrotica vittata* (Fabr.), retains the virus of cucumber mosaic for a short time only. They found that the virus-free melon aphid, *Aphis gossypii* Glover, could become infective after only 5 minutes' feeding on a cucumber-mosaic plant and could produce infection after feeding on a healthy plant for a like period. Aphids from mosaic plants do not produce the disease after 6 to 8 hours' confinement in a glass tube, this period being approximately the same as that during which expressed juice remains infective.

Although normally an "incubation period" of 1 day or longer is required before the beet leafhopper, *Eutettix tenellus* (Baker), is able to cause curly-top infection, Severin[15, 18] has demonstrated that when a large number of noninfective insects is used, they may on rare occasions transmit curly top after feeding for a period of 10 minutes on a diseased beet and 10 minutes on a healthy plant during high temperatures in the greenhouse. Transmissions were also obtained under similar conditions within periods of ½, 1, 1½, 2, 3, 4, 5, and 6 hours if the insects were fed for one-half of these periods on a diseased plant and the other half on a

[6] Since this paper was written, a paper by Merrill and Ten Broeck has come to my attention. They report a multiplication of the virus of equine encephalomyelitis in the mosquito vector, *Aedes aegypti*. The virus was carried from mosquito to mosquito by serial passage through seventeen lots of insects and was demonstrated to be present in undiminished amounts in the final lot of mosquitoes tested. (Merrill, M. H., and Carl Ten Broeck. The transmission of equine encephalomyelitis virus by *Aedes aegypti*. Jour. Exp. Med. **62**:687–95. 1935.)

healthy plant. The extract made from the mouth parts of leafhoppers which had fed ½ to 1 hour on a diseased beet was found on rare occasions to be infective. He states, "These experiments indicate that contamination of mouth parts without multiplication of the curly-top virus in the body of the insect may account for the transmission of the disease."

Swezy[27] proposed two theories to account for the transmission of curly top during short periods by the beet leafhopper: (1) an abnormal conditon of the alimentary canal consisting of a clump of bacteria in the lumen of the esophagus anterior to the esophageal valve might hinder the free passage of food, and infected beet juice might, therefore, be regurgitated through the mouth parts a short time after being taken in; (2) the passage of the infective organism unchanged through the body of the insect to the salivary glands and its expulsion in the saliva to cause infection. A third theory proposed "that a change in life cycle of the infective organism occurs in the body of the insect and this must be completed before the insect is capable of readily transmitting the disease to a healthy plant."

Period of Delay in Development of Infective Capacity in the Insect.—Severin[18] defined the virus "incubation period" in the beet leafhopper as "the time for the infective principle to pass into the mouth parts, alimentary canal, blood, salivary glands and out of the mouth parts in sufficient quantity to produce infection." He found that a low percentage of single leafhoppers were able to acquire and transmit the virus under the high temperatures in the greenhouse, during as short a period as 7 hours. Infections were also produced by single insects after periods varying from 9 to 23 hours.

Smith and Boncquet[22] reported that approximately 3 hours of feeding were necessary before the insect could obtain the infective agent and that an "incubation period" of from 24 to 48 hours was necessary before the beet leafhopper could transmit curly top of sugar beets. These facts suggested to them that curly-top virus was not merely transferred by a mechanical process, but that some development or change took place within the body of the insect during the first few hours after feeding on a diseased plant.

Carsner and Stahl[6] found that single beet leafhoppers were able to transmit curly top within a period of 21¾ hours after first feeding on a diseased beet. They reported that "a greater number of insects become able to transmit the virus after a longer period than 24 hours than are able to do so in the shorter time. The facts cited seem to indicate that a multiplication of the causal agent takes place within the insects."

Kunkel,[11] working in New York, demonstrated that the "incubation

period" of the aster-yellows virus in *Cicadula divisa* Uhl. [*C. sexnotata* (Fall.)] varied from 10 to 19 days. He also showed[12] that this period varied from 17 to 26 days with the California aster-yellows virus in the same species of leafhopper. He states, "It is difficult to conceive that any agent other than a living organism would require an incubation period in the insect carrier."

Bald and Samuel[1] showed that in the transmission of spotted wilt of tomato there was a delay of 5 to 7 days in the development of the infective capacity of the black carnation thrips, *Frankliniella insularis* (Franklin).

In his work with yellow spot of pineapple, Linford[13] reported that a period of approximately 10 days must elapse after onion thrips, *Thrips tabaci* Lindeman, have fed on a diseased plant before they become infective.

Elze[9] demonstrated that the period between the first feeding on a diseased leafroll potato plant and the development of infectivity in the peach aphid, *Myzus persicae* (Sulzer), ranged from 24 to 38 hours.

Smith[20] considered the minimum "incubation period" of the virus of leafroll of potato in the peach aphid to be 54 hours.

Storey[24] found that the "incubation period" of the virus of streak of maize in the leafhopper, *Cicadulina mbila* (Naude), was from 6 to 63 hours at 30° C. In some experiments 84 hours elapsed before the previously noninfective insects produced an infection after having first fed on a diseased plant. He states, "There was a great irregularity in the behavior of individual leafhoppers which makes impossible any exact numerical expression of the duration of the uninfective period."

Retention of Plant Virus by Insects.—The fact that insect vectors of viruses often retain their infective power over long periods of time has been considered as evidence in favor of the theory that a virus multiplies in the body of the insect. According to the literature, there are, however, some insect vectors of viruses which lose the infective capacity when they do not have access to a source of virus.

Boncquet and Stahl[4] asserted that the ability of the beet leafhopper to transmit curly top was lost in 15 to 35 days if the insects were transferred daily to a healthy beet.

Carsner[5] stated that infective beet leafhoppers after being kept on cattle spinach, *Atriplex polycarpa,* which is nonsusceptible to curly top, retained their infectivity for a period of 58 days.

Severin[16] found that three infective male beet leafhoppers infected in the first nymphal instar retained their infectivity during all of the nymphal stages and during their entire adult life of 101, 103, and 105

days, respectively. Eight infective males each provided with a healthy beet daily during its adult life transmitted the virus to from 16.2 to 55.5 per cent of the beets. These males usually transmitted curly top to fewer beets toward the end of their natural life.

Kunkel,[11] working in New York with aster yellows, showed that 3 of 6 adult *Cicadula divisa* retained their infective capacity for a period of 100 days when transferred at intervals to healthy asters. The other 3 insects did not infect any plants during the last 3 weeks of the experiment. He confined infective insects on rye plants, which are immune to aster yellows, for at least 2 months and demonstrated that the leafhoppers had not lost the infective principle during this period. He found, however, that while some specimens retained the infectivity throughout life, others seemed to lose it after a short time.

In Australia, Samuel, Bald, and Pittman[14] demonstrated that the black carnation thrips, the vector of spotted wilt of tomato, when placed on a healthy plant each day, did not lose the power to cause infection during the 24 days of the experiment, which included the pupal period. The insects, however, did not infect every plant on which they fed.

Elze[9] found that the peach aphid, the vector of leafroll of potato, does not lose its power to infect during the process of molting. When infective aphids were transferred to a nonsusceptible host plant such as spinach for 7 to 10 days, they were able to infect healthy potatoes.

Smith[20] reported that infective peach aphids retained the virus of leafroll of potato for a period of 7 days while feeding on immune plants such as cabbage. He said, "It seems probable, but this has not been proved, that *Myzus persicae* once infected remains so for the rest of its natural life."

In the case of curl of raspberry, Bennett[2] stated that the aphid vector, *Aphis rubiphila* Patch, does not lose its power to infect when kept on plants immune to curl for a period of 3 weeks and probably for the life time of the insect.

Bennett[3] reports that the aphid vector of red-raspberry mosaic, *Amphorophora rubi* (Kaltenbach), acquired the virus in 12 hours or less and transmitted it to healthy plants during the following 12-hour period, but when this aphid was transferred to successive healthy plants, it lost the mosaic virus.

Storey[24] showed that three single specimens of *Cicadulina mbila* retained the power to transmit the virus of streak of maize for periods of at least 84, 111, and 116 days, when provided with a healthy maize plant daily. One of eight insects tested, however, after infecting 16 of the first 33 plants, showed a progressive weakening of the infective power, and it

infected plants, thereafter, only on the 42nd, 45th, and 66th day and failed to produce an infection during the last 45 days of its life. The author states that "some specific biological relationship exists between *Cicadulina mbila* and streak virus. The evidence clearly indicates a multiplication of the virus in the insect."

Storey[20] later reported that insects infected by feeding upon streak-diseased maize normally continued to cause infections up to the time of their death. However, insects infected by inoculating them with streak virus by means of a needle puncture in their abdomen, ultimately became noninfective if kept on healthy plants. Plants infected by insects inoculated with the virus by the needle-puncture method developed symptoms of the disease more slowly than those infected by insects which had fed on diseased plants. Insects which had lost the power to cause infection were reinfected either by feeding them on a diseased plant or by needle inoculation, and they were then capable of again causing infection. Storey stated, "On the whole, the available evidence rather suggests that the loss is due to an exhaustion of the supply of virus in the insect."

An investigation was undertaken to determine whether the curly-top virus multiplies in the beet leafhopper. Since we have been unable to carry out experiments which would give direct evidence regarding multiplication of the virus in the leafhopper, an attempt was made to perform experiments which could give indirect evidence on this question. Such evidence might be obtained if it could be shown whether the insects retained the infective capacity during their entire adult life, and whether leafhoppers fed for short periods on a diseased beet could cause as many infections as those fed for longer periods on a source of virus. Transmission experiments were performed to ascertain the number of infections produced, and the longevity of the virus in the leafhopper during adult life, after having fed for varying periods on curly-top beets. Experiments were also conducted on the transmission of curly top by male and female leafhoppers, the ability of the leafhopper to acquire and transmit the virus during various periods of adult life, and whether an insect could be reinfected with the virus during later adult life. A comparison was made of the incubation period of the disease in sugar beets infected by the insects fed for short periods with those fed during the nymphal stages on curly top beets.

GENERAL METHODS

In all transmission experiments in which the beet leafhoppers were transferred daily to healthy beets, each specimen was confined in a small cylindrical cage 7½ inches in height by 5½ inches in diameter constructed of wood with top and sides covered with lawn except for a glass plate 3 by 6 inches through which observations were made (fig. 1). The bottom of the cage was made of wood and had a small circular opening 1 inch in diameter through which the beet seedling was inserted. The wooden frame and bottom of the inside of the cage was painted black so that the adults could readily be observed during the transfers. To prevent the escape of the insect from the cage enclosing the beet seedling, the soil in the pots was covered with a layer of coarse dry sand and the bases of the petioles were surrounded with cotton (fig. 1, *C*). The adults were transferred from one cage to another by capturing them with a glass pipette (fig. 2).

Curly-top beets used as a source of virus in the experiments reported in this paper were severely affected and were obtained from the San Joaquin Valley and interior regions of the Salinas Valley. During the four seasons 1931–1935 in which these experiments were performed, the beet leafhoppers were always infected with the virus by feeding them on curly-top beets in an advanced stage of the disease. Reliable symptoms of curly top, such as cleared veinlets and protuberances on the lower surface of the leaves, were present on all beets used as a source of virus in the various experiments.

Healthy sugar-beet seedlings with from 6 to 12 leaves were used in all experiments. The beet seedlings were grown in 4-inch clay pots and were kept out of doors in insect-proof cages.

The infective beet leafhoppers were reared during their nymphal stages on curly-top beets, the nymphal period requiring from 26 to 36 days in the greenhouse, as determined by Severin.[17]

Noninfective beet leafhoppers were obtained by transferring recently hatched nymphs before feeding from a diseased to a healthy sugar beet by means of a camel's-hair brush, as first described by Stahl and Carsner.[23] A supply of noninfective leafhoppers was reared on healthy beets in large cages in the greenhouse. The healthy beets were kept in insect-proof cages to protect them from accidental infection with curly top. Each cage of noninfective leafhoppers was numbered and a record was kept of the number on the cage from which the insects were taken. The noninfective leafhoppers in each cage were tested at monthly intervals

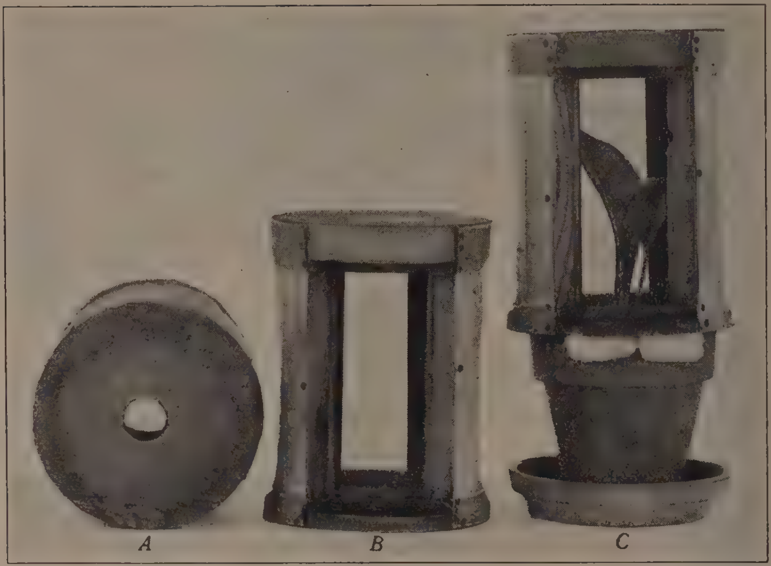

Fig. 1.—View of cages used in determining the period of infectivity during the adult life of the beet leafhopper. *A*, Bottom board with circular hole through which the leaves of a beet seedling projected into the cage; *B*, cage with top and sides covered with lawn, except a glass plate through which observations were made; *C*, cage enclosing a beet seedling with the base of the petioles surrounded with cotton.

Fig. 2.—Pipettes with a capacity of 10 cc used in capturing beet leafhoppers. *A*, Pipette with a piece of silk bolting covering the opening between the pipette and rubber tube; *B*, pipette, silk bolting, and rubber tube. The end of the rubber tube is held in the mouth. By inhaling a breath of air through the rubber tube, the leafhoppers are drawn into the bulb of the pipette, and by exhaling the breath and snapping the first two fingers against the side of the pipette they are expelled.

by transferring 5 to 10 specimens to a healthy beet seedling. If the noninfective insects in a cage accidentally became infected with curly top, the results with leafhoppers from such a cage were rejected.

Recently molted adults were used in all experiments for two reasons: (1) the insects feed for long periods of time after the last molt and, therefore, more frequently become infective when feeding on a diseased beet, and (2) the age of the adult from the time it acquires the winged stage is known. The long feeding periods may be explained by the fact that before molting the insect stops feeding and empties the contents of the alimentary tract.

To avoid injury, the recently molted adults were allowed to rest for a period of time on the nymphal skins until the wings expanded and the chitin was somewhat hardened. The adults were then captured with a pipette and transferred to an empty cage in which they were confined for a period varying from 2 to 4 hours before feeding them on a curly-top beet.

Each recently molted noninfective adult was transferred from the empty cage with a pipette, gently dropped on a diseased beet leaf, and observed as it fed for short periods. The insect usually inserted its mouth parts into the plant tissue, as could readily be seen by use of a reading glass. Sometimes the leafhopper would withdraw its mouth parts to seek a more suitable place to feed, often on the lower surface of the leaf. In the majority of cases, however, once the insect began to feed, its mouth parts were rarely withdrawn during short feeding periods until interrupted in its meal. During the short feeding periods on curly-top beets a high temperature of approximately 100° F was maintained in the greenhouse. When the leafhopper had fed for the desired length of time, it was interrupted in its meal and transferred to a healthy beet seedling confined in a cage.

Each adult in a cage was transferred at intervals of 24 hours to successive healthy beets. The transfers were made by jarring the cage on the top of the table and by blowing a breath of air through the lawn covering of the cage to cause the leafhopper to hop from the beet seedling to the lawn, wooden frame, or bottom of the cage. The beet was removed by lifting the cage from the clay pot and by pulling the beet leaves through the opening in the bottom. The hand was placed over the opening to prevent the escape of the insect. The cage containing the leafhopper was then placed over another healthy beet.

The beet seedlings, after exposure to the leafhoppers for the desired length of time, were placed in insect-proof cages in the greenhouse. The beets were examined daily for a period of 6 weeks for symptoms of curly

top. If any of the beets developed cleared veinlets on the youngest leaves, the leafhopper had evidently infected the plant during the 24-hour period that it had been confined on the beet.

Repeated capturing of the insects with a pipette was necessary in transferring them from one cage to another in the experiments on the loss of the infective capacity by the leafhoppers when kept on a plant immune to curly top. This resulted in a high mortality, and hence some of the experiments could not be extended over as long a period of time as was desired. In most other experiments in which the leafhoppers were transferred from one plant to another in cages, they were rarely captured by means of the pipette and lived for long periods of time.

The sexes were segregated soon after molting when large numbers of beet leafhoppers were required for an experiment. A large number of last-instar nymphs were usually captured and confined in a cage, and after they passed through the last molt, the male and female leafhoppers were transferred to separate cages. This procedure was followed to prevent fertilization of the females, since eggs deposited by fertilized females would hatch nymphs in the insect-proof cages, which would introduce a source of error into the results.

TRANSMISSION OF CURLY TOP BY BEET LEAFHOPPERS FED TEN MINUTES TO THREE HOURS ON A DISEASED BEET

If there is a multiplication of the virus in the insect, those which have fed for short periods of time on curly-top beets should, after the "incubation period" is completed, be able to cause as many infections as those fed for longer periods of time. The following experiment was therefore designed to determine whether a multiplication of the curly-top virus takes place in the leafhopper, or whether the insect only accumulates the virus during repeated feedings and is in reality only an internal mechanical carrier.

Recently molted, previously noninfective leafhoppers were fed for periods of 10, 20, 40, 60, 120, and 180 minutes on a diseased plant and were than transferred to successive healthy beets at intervals of 24 hours during their adult life. If no infections resulted from the feeding of the previously noninfective leafhoppers fed for short periods on a diseased beet during the first 30 days, they were considered noninfective and transfers to successive healthy beets were discontinued. Often, however, after the insects had been discarded, one or more of the beets inoculated by such specimens would develop symptoms of the disease, which indicated that the insects had been infective.

Severin[15] has demonstrated that noninfective beet leafhoppers were able to recover the curly-top virus from beet seedlings with from 4 to 6 leaves 2 days after infective insects were first fed on them. The cleared veinlets sometimes appeared on the youngest leaves at the end of 2 days, but noninfective insects did not always become infective by feeding on seedlings showing this early symptom. These results indicate that leafhoppers can recover virus by feeding for several days on plants which they have infected. This fact has not always been considered in transmission experiments in which an attempt was being made to determine how long the insect vector retained the infective capacity. The feeding period of the leafhoppers in the present experiments was only 24 hours on each beet, and it was considered impossible for them to recover any virus which they had inoculated into and which had multiplied in the beet during the period they were confined on the seedling.

TABLE 1

INFECTION OF BEET LEAFHOPPERS WITH CURLY TOP BY FEEDING FOR SHORT PERIODS ON DISEASED BEETS

Period on curly-top beet, minutes	Number fed on curly-top beets	Number infective	Number not infective	Per cent infective
10	16	7	9	43.7
20	18	7	11	38.9
40	11	7	4	63.6
60	11	7	4	63.6
120	6	3	3	50.0
180	13	7	6	53.9
Total	75	38	37	....
Average	..	..	..	50.8

Seventy-five recently molted, previously noninfective leafhoppers were fed for short periods on diseased beets, as shown in table 1, and of this number 38 acquired and transmitted the virus to beets, while 37 failed to become infective. The results in table 1 suggest that there was a slight increase in the percentage that acquired the infective power as the length of the feeding periods was increased. The data indicate that 50.8 per cent of the insects allowed to feed for short periods on a diseased beet became infective.

The results in table 2 show a great variation in the delay in the development of the infective capacity in single beet leafhoppers. The period of time that elapsed between the initial feeding on a curly-top beet and the first infection by the leafhopper varied from 1 to 44 days, or an average of 9.6 days when the insects were kept at temperatures varying

TABLE 2

Transmission of Curly Top by Single Beet Leafhoppers Fed from 10 to 180 Minutes on a Diseased Beet and on a Healthy Beet Daily During Adult Life

Period on curly-top beet, minutes	Sex	No.	Dates fed on healthy beets	Beets inoculated	Beets infected		Period of delay in development of infective capacity, days	Adult age when last infection produced, days	Longest period between two infections, days	Period between last infection and death of insect, days	Per cent of total infections during					
					Number	Per cent					1–30 days	31–60 days	61–90 days	91–120 days	121–150 days	151–180 days
10	♀	1	July 8–Aug. 15	39	1	2.6	36	36	...	3	0.0	100.0	....	....	....	....
		2	Sept. 7–Oct. 25	49	1	2.0	44	44	...	5	0.0	100 0	....	....	....	....
		3	Sept. 4–Nov. 19	77	2	2.6	15	50	35	27	50.0	50.0	0.0	....	....	....
		4	May 8–July 30	83	4	4.8	9	32	12	51	75.0	25.0	0.0	....	....	....
	♂	1	June 25–Aug. 19	55	4	7.3	1	31	21	24	75.0	25.0	....	....	....	....
		2	Sept. 3–Dec. 20	108	3	2.8	5	81	43	27	33 3	33.3	33.3	0.0	....	....
20	♀	5	July 8–Aug. 28	52	3	5.8	7	45	35	7	33.3	66.7	....	....	....	....
		6	Sept. 6–Mar. 9	183	1	0.5	1	1	..	182	100.0	0.0	0.0	0.0	0.0	0.0
	♂	3	June 25–Aug. 8	44	1	2.3	15	15	...	29	100.0	0.0	....	....	....	....
		4	July 24–Oct. 27	95	6	6.3	1	71	24	24	83 3	0.0	16.7	0 0	....	....
		5	June 3–Oct. 29	150	2	1.3	7	117	110	33	50.0	0.0	0.0	50.0	0.0	....
40	♀	7	July 7–Sept. 3	58	1	1.7	7	7	...	51	100.0	0.0	....	....	....	....
		8	June 3–Dec. 5	184	4	2.2	10	161	105	23	25.0	0.0	0.0	25.0	25.0	25.0
	♂	6	May 7–July 28	83	5	6.0	6	34	10	49	80.0	20.0	0.0	....	....	....
		7	June 25–Nov. 4	132	2	1.5	8	17	8	115	100.0	0.0	0.0	0.0	0.0	....
60	♀	9	May 25–July 25	61	3	4.9	10	18	10	43	100.0	0.0	....	....	....	....
		10	July 7–Oct. 27	111	3	2.7	7	18	7	93	100.0	0.0	0.0	0.0	....	....
		11	May 7–Aug. 18	103	2	1.9	4	21	16	82	100.0	0.0	0.0	0.0	....	....
		12	July 7–Jan. 4	180	7	3.9	7	130	77	50	42.9	0.0	0.0	28.6	28.6	0.0
	♂	8	June 3–Nov. 10	160	5	3.1	8	68	45	92	80.0	0.0	20.0	0.0	0.0	0.0
120	♀	13	May 7–Sept. 1	117	6	5.1	9	77	25	40	33.3	33.3	33.3	0 0	....	....
	♂	9	June 3–July 28	55	5	9.1	3	25	9	30	100.0	0.0	....	....	....	....
180	♂	10	June 25–Sept. 15	82	1	1.2	1	1	...	81	100.0	0.0	0.0	....	....	....
		11	May 8–Aug. 14	98	3	3.1	9	32	21	66	66.7	33.3	0.0	0.0	....	....
		12	Sept. 6–Jan. 4	121	9	7.4	9	105	38	16	44.4	33.3	11.1	11.1	....	....
Average	......	..	....	99.2	3.4	3.4	9.6	49.5	34.3	49.7	*7.2**	*2.2**	*1.3**	*1.6**	*1.8**	*1.0**

from 70° to 100° F in the greenhouse. The leafhoppers caused infections at very irregular intervals after the first infection.

The leafhoppers fed for short periods of from 10 to 180 minutes on a curly-top beet were able to cause only a few infections. As shown in

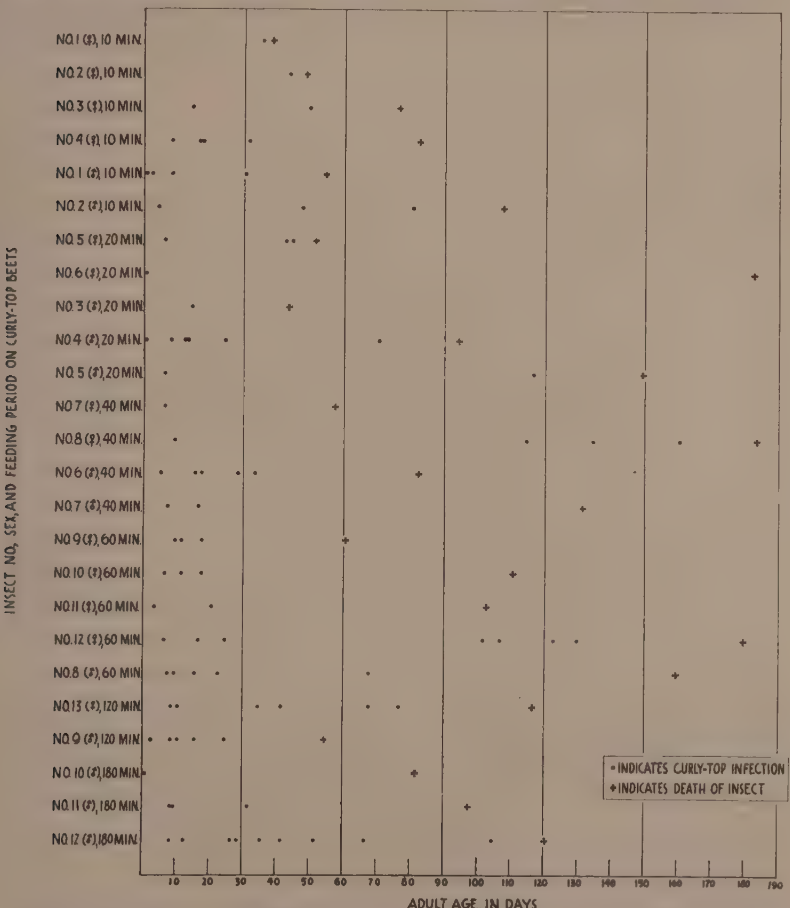

Fig. 3.—Number and distribution of curly-top infections produced by single beet leafhoppers fed for short periods varying from 10 to 180 minutes on diseased beets and transferred daily to a healthy beet. The insects produced most of the infections during the first 30 days of adult life, after which there was a decrease in the infective capacity of the insects.

table 2, six leafhoppers fed for a period of 10 minutes on a diseased beet infected from 1 to 4 beets, five fed 20 minutes infected from 1 to 6 beets, four fed 40 minutes infected from 1 to 5 beets, five fed 60 minutes infected from 2 to 7 beets, two fed 120 minutes infected from 5 to 6 beets,

and three fed 180 minutes infected from 1 to 9 beets during adult life. The insects fed for short periods on curly-top beets averaged 3.4 infections when each was provided with a healthy beet seedling daily during its adult life.

The data in table 2 indicate that the leafhoppers were slightly infective throughout life, but that they were highly infective only during the first 30 days of adult life. The age of the adults when the last infection was produced varied from 1 to 161 days, and averaged 49.5 days. The number of beets inoculated, which also represents the longevity of the leafhoppers in days, varied from 39 to 184 and averaged 99.2. The maximum period between any two infections varied from 7 to 110 days and averaged 34.3 days, while the number of days between the last infection and the death of the insect varied from 3 to 182 days and averaged 49.7 days. The data show an average of 7.2 per cent of the beets were infected during the first 30 days of adult life. There was a marked decline in the number of infections during the second 30-day period to an average of 2.2 per cent and a further decrease during the third 30-day period to an average of 1.3 per cent. After these periods the percentage of beets infected remained approximately constant until the death of the insect.

Figure 3 shows the frequency and distribution of curly-top transmissions by beet leafhoppers fed for short periods on diseased beets and indicates that a majority of the infections are caused during the first 30 days of adult life. The results indicate that after this period the frequency of curly-top infections by the leafhopper becomes less until the ninetieth day, after which the frequency of infections remains approximately constant. After the period of delay in the development of the infective capacity, there are no indications of any cycles of development nor any evidence that the virus multiplies in the insect vector. Some leafhoppers apparently retained the infective capacity during their entire adult life, as shown by female No. 8 and male No. 12. Others, such as female No. 6 and male No. 8 apparently lost the power to cause infection during later life, as shown in figure 3.

TRANSMISSION OF CURLY TOP BY BEET LEAFHOPPERS FED FROM SIX HOURS TO TWENTY-EIGHT DAYS ON DISEASED BEETS

In order to determine whether there was any direct relation between the length of time a beet leafhopper feeds on a diseased plant and the number of infections it produces during its adult life, 18 recently molted previously noninfective leafhoppers were fed for periods of ¼ (6 hours),

½ (12 hours), 1, 3, 7, 14, 21, and 28 days on two curly-top beets, 10 males on one and 8 females on the other. Each insect was then provided daily with successive healthy beets.

The results of the experiment show a great variation in the number of infections produced by the beet leafhoppers fed for different periods of

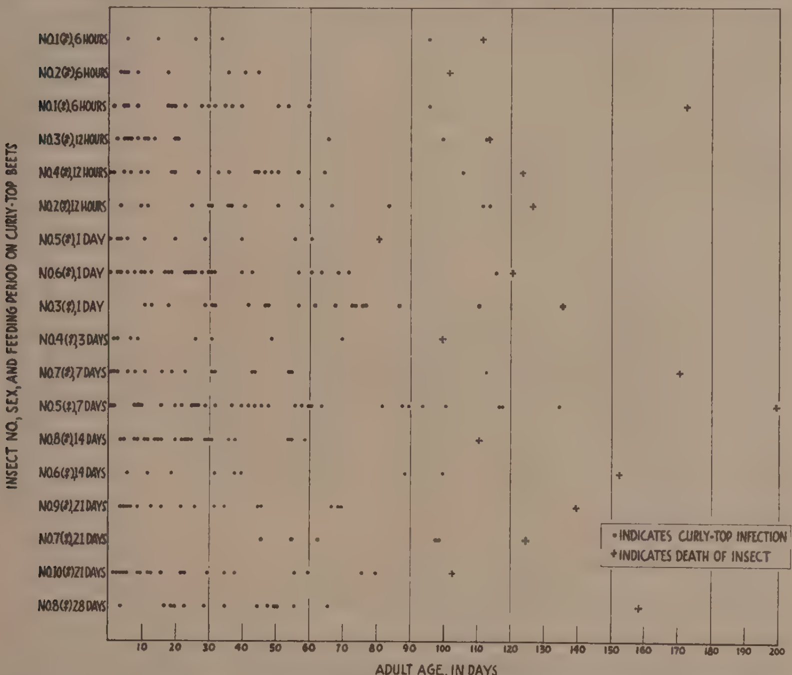

Fig. 4.—Number and distribution of curly-top infections produced by single beet leafhoppers fed from 6 hours to 28 days on a diseased beet and transferred daily to a healthy beet. Most of the infections occurred during the first 30 days of adult life. The infective power was apparently lost by some of the leafhoppers toward the end of their natural life, but others retained the infectivity up to the time of death.

time on curly-top beets. The number of beets infected by insects fed from 6 hours to 28 days on a curly-top beet varied from 5 to 32, or an average of 15.1, as shown in table 3. One specimen fed for 6 hours and another for 21 days on a curly-top beet produced only 5 infections, while a third insect fed for 7 days infected 32 beets during its adult life, as indicated in table 3. From 4.0 to 22.3 per cent, or an average of 11.6 per cent, of the beets were infected with curly top during the adult life of the leafhopper.

The data in table 3 also indicate that in the majority of the beet leafhoppers there was a decrease in the infective capacity during the later

TABLE 3

Transmission of Curly Top by Single Beet Leafhoppers Fed from 6 Hours to 28 Days on a Diseased Beet and on a Healthy Beet Daily During Adult Life

Period on curly-top beet, days	Sex	No.	Dates fed on healthy beets	Beets inoculated	Beets infected		Period of delay in development of infective capacity, days	Adult age when last infection produced, days	Longest period between two infections, days	Period between last infection and death of insect, days	Per cent of total infections during					
					Number	Per cent					1–30 days	31–60 days	61–90 days	91–120 days	121–150 days	151–180 days
1/4	♂	1	May 3–Aug. 22	112	5	4.5	6	96	62	16	60.0	20.0	0.0	20.0	....	....
		2	May 3–Aug. 12	102	8	7.8	4	45	18	57	62.5	37 5	0.0	0.0	....	....
	♀	1	May 3–Oct. 22	173	18	10.5	2	96	36	77	55 5	38 9	0.0	12.5	0.0	0.0
1/2	♂	3	May 3–Aug. 24	114	13	11.4	3	113	45	1	76.9	0.0	7.7	15.4	....	....
		4	May 3–Sept. 3	124	19	15 3	1	106	41	18	47.4	42.1	5.3	5.3	0.0	....
	♀	2	May 3–Sept. 6	127	15	11.8	4	114	28	13	33 3	40.0	13 3	13 3	0.0	....
1	♂	5	May 4–July 23	81	10	12.3	1	61	16	20	70.0	20.0	10.0	....	....	....
		6	May 4–Sept. 1	121	27	22 3	1	116	44	5	63.0	18.5	14.8	3.7	....	....
	♀	3	May 4–Sept. 16	136	18	13 2	11	111	24	25	22.2	33.3	38.9	5.5	0.0	....
3	♀	4	May 6–Aug. 13	100	8	8.0	2	70	21	30	62.5	25.0	12.5	0.0	....	....
7	♂	7	May 10–Oct. 27	171	16	9 4	1	113	58	58	56.3	37.5	0.0	6.3	0 0	0.0
	♀	5	May 10–Nov. 25	200	32	16.0	1	135	18	65	37.5	31.3	15 6	12 5	3 1	0.0
14	♂	8	May 17–Sept. 4	111	22	19.8	4	59	16	52	72 7	27.2	0.0	0.0	....	....
	♀	6	May 17–Oct. 15	153	8	5.2	6	100	48	53	37.5	37.5	25.0	0.0	0.0	....
21	♂	9	May 24–Oct. 10	140	16	11.4	4	70	21	70	56 3	25.0	18.7	0.0	0 0	....
	♀	7	May 24–Sept. 25	125	5	4.0	46	99	35	26	0 0	40.0	20.0	40.0	0.0	....
28	♂	10	May 31–Sept. 10	103	19	18.4	2	80	18	23	68.4	21.1	10.5	0.0	....	....
	♀	8	May 31–Oct. 5	159	13	8.2	4	66	13	93	46.1	46.1	7.7	0.0	0.0	0.0
Average	..	..	....	130 7	15.1	11.6	5.7	91.7	31.2	39	*26.5**	*15.0**	*5.6**	*3 9**	*0 5**	*0.0**

* Per cent beets infected.

stages of adult life. The age of the adults when the last infection was produced varied from 45 to 135 days, or an average of 91.7 days, while the longevity of the adults varied from 81 to 200 days, or an average of 130.7 days. As already stated, each insect was provided with a healthy beet daily during adult life, and hence the longevity of the adults in days corresponds to the number of beets inoculated in table 3. The longest period between two successive infections varied from 13 to 62 days, or an average of 31.2 days, while the period between the last infection and the death of the insect varied from 1 to 93 days, or an average of 39 days. In 11 of 18 specimens tested, the longest period between successive infections was less than the period between the last infection and the death of the insect; this fact suggests a decrease in the infective capacity of the insect.

The majority of leafhoppers produced more infections during the first 30 days of their adult life than during any succeeding 30-day period, as shown in figure 4. The percentage of the total beets infected during successive 30-day periods of adult life indicates a progressive decrease in the infective capacity, which suggests that the quantity of virus in the insect is being decreased as the insect feeds on consecutive healthy beets. The average percentages of beets infected during successive 30-day periods were as follows: 26.5, 15.0, 5.6, 3.9, and 0.5 respectively (table 3).

TRANSMISSION OF CURLY TOP BY BEET LEAFHOPPERS FED DURING NYMPHAL STAGES ON DISEASED BEETS

In connection with the preceding experiments on short feeding periods, it became desirable to determine if the length of feeding period on a source of virus would affect the number of infections produced. Accordingly, 10 recently molted adults which had fed on diseased beets during their entire nymphal stages were provided with successive healthy beets at 24-hour intervals during their adult life.

There was a great irregularity in the number of infections produced by the infective adults. These leafhoppers infected from 4 to 26 beets, or an average of 15.6 beets, during their adult life, as shown in table 4. They infected from 3.5 to 44.1 per cent, or an average of 11.8 per cent, of the beets on which they fed.

The age of the adults when they produced the last infection varied from 50 to 151 days, or an average of 81.3 days, while the number of beets inoculated, which also represents the longevity of the adults in days, varied from 58 to 206, or an average of 132.2. The longest period between two successive infections produced by a single specimen varied

TABLE 4

Transmission of Curly Top by Infective Beet Leafhoppers Fed During Nymphal Stages on a Diseased Beet and on a Healthy Beet Daily During Adult Life

Sex	No.	Dates fed on healthy beets	Beets inoculated	Beets infected		Adult age when last infection produced, days	Longest period between two infections, days	Period between last infection and death of insect, days	Per cent of total infections during					
				Number	Per cent				1–30 days	31–60 days	61–90 days	91–120 days	121–150 days	151–180 days
♀	1	Sept. 4–Oct. 31	58	10	17.2	50	13	8	80.0	20.0	....	....	....	....
	2	Sept. 4–Nov. 26	83	22	26.5	83	27	0	54.5	40.9	4.5	....	....	....
	3	Sept. 4–Nov. 28	87	11	12.6	59	15	28	63.6	36.4	0.0	....	....	....
	4	May 26–Sept. 15	113	4	3.5	80	47	33	50.0	25.0	25.0	0.0	....	....
	5	Aug. 24–Dec. 20	119	12	10.1	87	15	32	50.0	25.0	25.0	0.0	....	....
	6	Sept. 4–Jan. 25	144	22	15.3	67	20	77	72.7	18.2	9.1	0.0	0.0	....
	7	June 26–Dec. 11	168	18	10.7	50	12	118	83.3	16.7	0.0	0.0	0.0	0.0
	8	May 7–Oct. 27	173	17	9.8	108	25	65	47.1	11.8	35.3	5.9	0.0	0.0
	9	June 25–Dec. 31	189	15	7.9	104	42	85	26.7	60.0	0.0	13.3	0.0	0.0
	10	Sept. 4–Mar. 28	206	8	3.9	151	83	55	75.0	0.0	12.5	0.0	0.0	12.5
♂	1	May 26–July 24	59	26	44.1	59	7	0	61.5	38.5	....	....	....	....
	2	Aug. 24–Jan. 28	188	22	11.7	78	26	110	68.2	31.8	0.0	0.0	0.0	0.0
Average	......	....	132.2	15.6	11.8	81.3	27.7	50.9	*31.9**	*14.8**	*5.2**	*1.2**	*0.0**	*0.8**

* Per cent beets infected.

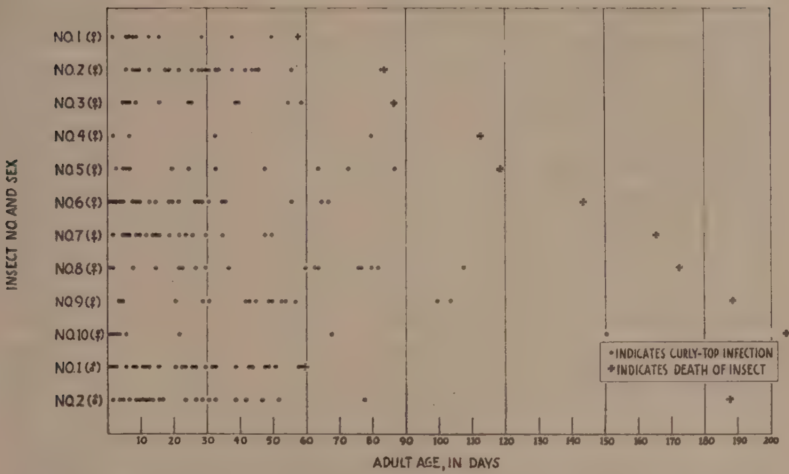

Fig. 5.—Frequency and distribution of curly-top infections by single beet leafhoppers that had completed the nymphal stages on diseased beets and were transferred daily to healthy beets during adult life. Some of the leafhoppers apparently lost the infective power during late adult life. Most of the infections were caused during early adult life.

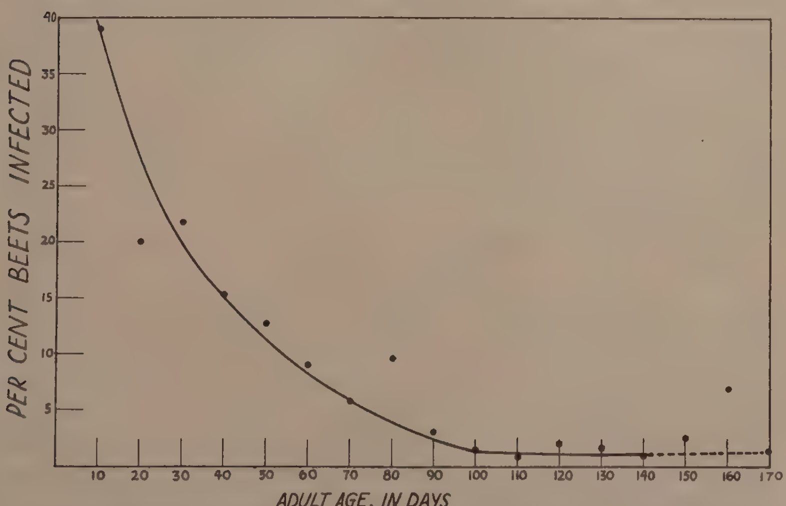

Fig. 6.—Decrease in infective capacity of the beet leafhopper as indicated by percentage of beets infected during successive 10-day periods of adult life. The nymphal stages were completed on curly-top beets and the adults were provided daily with a healthy beet until the end of their natural life.

from 7 to 83 days, or an average of 27.7 days, while the period between the last infection and the death of the insect varied from 0 to 118 days, or an average of 50.9 days (table 4). These results indicate a decrease in the power of the leafhoppers to cause infection as they approach old age.

As in preceding experiments with adult beet leafhoppers fed for varying periods on a curly-top beet, the insects bred as nymphs on curly-top beets also infected more beets during the first 30 days of their adult life than during any succeeding 30-day period. The average percentages of beets infected during successive 30-day periods of adult life (table 4) were as follows: 31.9, 14.8, 5.2, 1.2, 0.0, 0.8. These data indicate a gradual decrease of the infective capacity. The decrease and the fact that most beets are infected during early adult life of the insect are shown in figure 5, which gives the distribution of the infections caused by each beet leafhopper. Most of the beets were infected during early adult life, and as the insects grew older the frequency of the infections became less until finally most leafhoppers were no longer capable of causing infection. Several of the specimens failed to infect a beet for a long period of time and then suddenly produced several infections, as shown in figure 5 by female No. 9, but this was not usually the case. The leafhopper did not produce the disease in any characteristic cycles of infectivity, such as might be expected if the virus multiplied or passed through some sort of a cycle of development in the insect.

The gradual decrease of the infective capacity of the beet leafhopper is also illustrated by the curve in figure 6, which shows the percentage of beets on which the insects fed that were infected during successive 10-day periods of adult life.

TRANSMISSION OF CURLY TOP BY MALE AND FEMALE LEAFHOPPERS

A comparison was made of the transmission of curly top by male and female beet leafhoppers to determine if one of the sexes might not be a more efficient vector of the virus than another. If one sex proved more efficient in the transmission of the disease, better results would be obtained from experiments which were carried out with such individuals.

A lot of 8 male and 8 female leafhoppers that had completed the nymphal stages on the same diseased beet were each transferred to a healthy beet soon after completing the last molt. The single insects were transferred to successive healthy beets at intervals of 24 hours during the remainder of their adult life.

As shown in table 5, the males infected from 5 to 50 beets, or an aver-

TABLE 5

TRANSMISSION OF CURLY TOP BY SINGLE BEET LEAFHOPPERS FED DURING NYMPHAL STAGES ON A DISEASED BEET AND ON A HEALTHY BEET DAILY DURING ADULT LIFE

Sex	No.	Dates fed on healthy beets	Beets inocu-lated	Beets infected		Adult age when last infection produced, days	Longest period between two in-fections, days	Period between last in-fection and death of insect, days	Per cent of total infections during					
				Num-ber	Per cent				1–30 days	31–60 days	61–90 days	91–120 days	121–150 days	151–180 days
♂	1	Sept. 19–Feb. 18	153	10	6.5	123	52	29	50.0	20.0	20.0	0.0	10.0	0.0
	2	Sept. 19–Dec. 27	100	12	12.0	84	18	16	58.3	25.0	16.7	0.0	....	....
	3	Sept. 19–Feb. 10	145	15	10.3	75	20	70	40.0	46.7	13.3	0.0	0.0	....
	4	Sept. 19–Feb. 25	160	5	3.1	125	60	23	40.0	20.0	20.0	0.0	20.0	0.0
	5	Sept. 18–Dec. 4	77	12	15.6	77	16	0	50.0	33.3	17.6	....	....	....
	6	Sept. 18–Apr. 15	209	15	7.2	148	73	61	66.7	6.7	20.0	0.0	6.7	0.0
	7	Sept. 18–Apr. 7	201	50	24.9	158	21	43	42.0	20.0	22.2	4.0	6.0	6.0
	8	Sept. 18–Nov. 21	64	14	21.9	56	24	8	78.6	21.4	0.0	....	....	....
	Av.	....	138.6	16.6	12.0	105.7	35.5	31.2	*28.5**	*12.9**	*11.4**	*1.8**	*4.1**	*4.1**
♀	1	Sept. 19–Apr. 5	199	18	9.0	164	38	35	38.9	22.2	16.7	5.5	5.5	11.1
	2	Sept. 19–Jan. 6	110	5	4.5	62	36	48	80.0	20.0	0.0	0.0		
	3	Sept. 19–Feb. 14	149	12	8.0	146	70	3	41.7	33.3	16.7	0.0	8.3	....
	4	Sept. 19–Mar. 31	194	15	7.7	78	23	116	60.0	33.3	6.7	0.0	0.0	0.0
	5	Sept. 18–Mar. 27	190	15	7.9	167	69	23	53.3	20.0	0.0	6.7	6.7	13.3
	6	Sept. 18–Mar. 8	171	3	1.7	63	46	108	33.3	33.3	33.3	0.0	0.0	0.0
	7	Sept. 18–Feb. 26	161	13	8.1	159	49	2	46.2	15.2	0.0	15.2	0.0	23.1
	8	Sept. 18–Jan. 5	109	5	4.6	10	4	99	100.0	0.0	0.0	0.0	....	....
	Av.	....	160.4	10.7	6.7	106.1	41.9	54.2	*18.7**	*7.9**	*3.3**	*1.8**	*1.7**	*5.7**

* Per cent beets infected.

age of 16.6 beets, during their adult life. The females infected from 3 to 18 beets, or an average of 10.7 beets, during their adult life. The males infected an average of 12.0 per cent, while the females infected an average of 6.7 per cent of the beets on which they fed. The results of this experiment seem to indicate that the males have a slightly greater ability

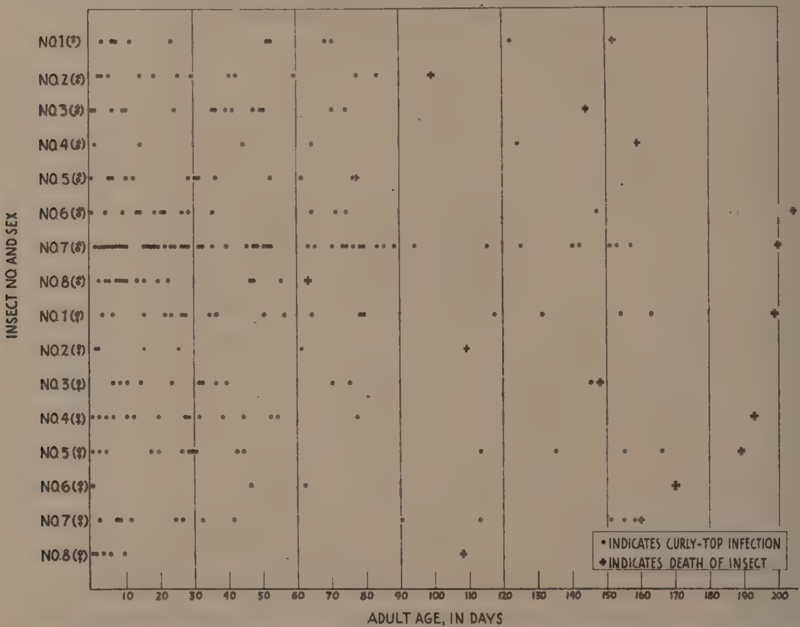

Fig. 7.—Number and distribution of curly-top infections produced by 8 male and 8 female beet leafhoppers which had completed the nymphal stages on the same diseased beet and were transferred daily to healthy beets during their adult life. Most of the infections occurred during the first 30 days of adult life, and, while some insects apparently lost the infective capacity as they approached old age, others retained the power to cause infection.

to transmit the virus. The great variation in the number of beets infected by single male and female adults, however, precludes any definite conclusion.

The results in table 5 indicate that the two sexes retained the power to transmit the virus for approximately the same length of time. The age of the males when they produced the last infection averaged 105.7 days, while the age of the females averaged 106.1 days when they produced the last infection. The longevity (corresponding with the number of beets inoculated) of the females in days was greater than that of the males, averaging 160.4, while the males averaged 138.6 (table 5).

The distribution of the beets infected during the adult life of the two

sexes was very similar. The males infected an average of 28.3 per cent and the females 18.7 per cent of the beets they fed on during the first 30 days of adult life (table 5). During the period from 31 to 60 days, the males infected an average of 12.9 per cent and the females an average of 7.9 per cent of the beets. After a continued decrease during the next 30-day period, as shown in table 5, the average infective capacity of the two sexes remained approximately constant.

Figure 7 illustrates the distribution of infections by the eight male and eight female adults and shows that most of the infections were produced during early adult life. Some of the insects, for example, male No. 3 and females No. 4 and No. 8, failed to infect beets toward the end of their natural life; this indicates that they had apparently lost the infective capacity, while other specimens, such as females No. 3 and No. 7 retained their infectivity up to the time of their death.

LOSS OF INFECTIVE CAPACITY BY LEAFHOPPERS KEPT ON PLANT IMMUNE TO CURLY TOP

Experiments were conducted to determine whether infective beet leafhoppers would lose the infective power when kept on a plant immune to curly top, such as Alameda or Mammoth sweet corn (*Zea mays*). In three different experiments, one hundred recently molted adults which had completed their nymphal stages on a diseased beet were transferred singly to healthy beets for a period of 24 hours to determine the percentage of insects that would transmit curly top during that period. The leafhoppers were fed on sweet corn for a period of 9 days, and then on the 10th day they were transferred singly to healthy beet seedlings for 24 hours. The procedure was repeated several times up to the 90th day of the adult life, when the number of insects was reduced so low that further tests were impossible owing to the high mortality caused by the repeated capture of the insects with the pipette.

The results of the three experiments indicate a gradual loss of the infective capacity by the leafhopper when confined on sweet corn. Table 6 summarizes the results of the three experiments and shows that 50.8 per cent of the insects transmitted curly top during the first transfer to healthy beets. On the 10th day 55.5 per cent of the healthy beets became diseased, after which time, with succeeding 10-day periods, there was a drop in the percentage of leafhoppers that transmitted curly top, until the 80th day when only 3 per cent, and on the 90th day 3.1 per cent, were still able to cause infection. The data obtained in these three experiments are plotted in figure 8, which shows the loss of the infective capacity by the leafhoppers when confined on sweet corn.

TABLE 6

LOSS OF INFECTIVE CAPACITY BY BEET LEAFHOPPERS KEPT ON SWEET CORN AND TRANSFERRED EVERY TENTH DAY TO A HEALTHY BEET

Days on sweet corn	Females		Males		Per cent infected
	Beets inoculated	Beets infected	Beets inoculated	Beets infected	
Experiment No. 1					
1	50	30	50	29	59.0
10	50	31	50	36	67.0
20	50	17	50	26	43.0
30	50	9	50	19	28.0
40	65	24	35	14	38.0
50	42	4	13	1	9.1
60	29	0	3	2	6.6
70	21	4	..	..	19.0
80	16	1	..	..	6.3
90	..	..	..	..	....
Experiment No. 2					
1	50	20	50	18	38.0
10	25	15	25	12	54.0
20	25	8	25	15	46.0
30	25	4	25	9	26.0
40	25	5	25	3	16.0
50	23	0	25	1	2.1
60	13	1	22	3	11.0
70	7	2	7	1	21.0
80	..	..	..	..	....
90	..	..	..	..	....
Experiment No. 3					
1	..	..	50	30	60.0
10	..	..	50	17	34.0
20	..	..	50	17	34.0
30	..	..	50	8	16.0
40	..	..	50	9	18.0
50	..	..	50	8	16.0
60	50	2	11	0	3.3
70	50	4	..	..	8.0
80	50	1	..	..	2.0
90	32	1	..	..	3.1
Results of 3 experiments summarized					
1	100	50	150	77	50.8
10	75	46	125	65	55.5
20	75	25	125	58	41.5
30	75	13	125	36	24.5
40	90	29	110	26	27.5
50	65	4	88	10	9.2
60	92	3	36	5	6.3
70	78	10	7	1	12.9
80	66	2	..	..	3.0
90	32	1	..	..	3.1

The results of the three preceding experiments demonstrate that the beet leafhopper gradually loses its infective capacity when not given the opportunity to become reinfected by feeding on a curly-top beet. The leafhopper may gradually exhaust its store of virus by giving off a certain quantity of virus in the saliva during feeding.

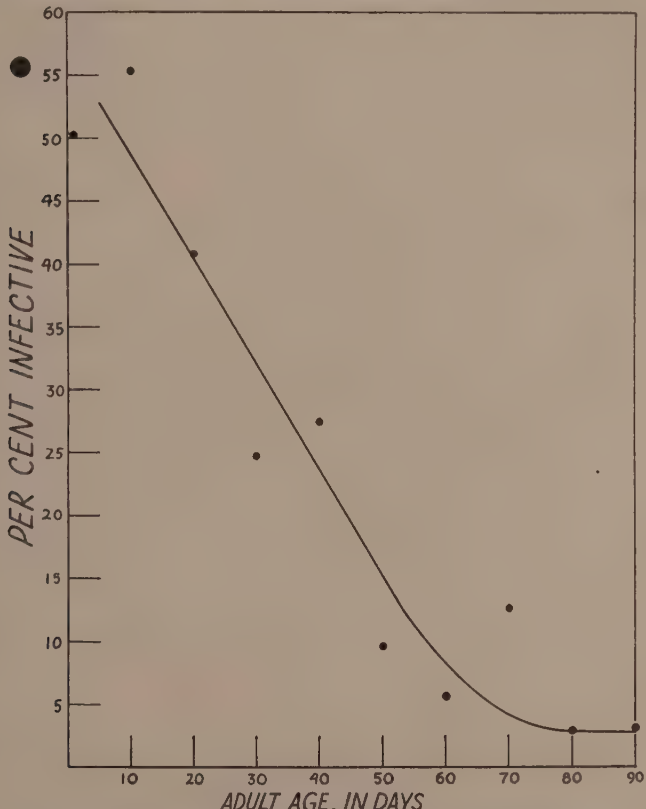

Fig. 8.—Percentage of beet leafhoppers which were infective during successive 10-day periods of adult life. The nymphal stages were completed on diseased beets and the adults were fed successively on sweet corn for 9 days and on healthy beets for 1 day during their entire adult life.

ABILITY OF BEET LEAFHOPPER TO ACQUIRE AND TRANSMIT CURLY TOP DURING ADULT LIFE

Two explanations were considered possible for the fact that the infective beet leafhopper caused fewer infections as it approached old age than during early adult life. The insect may gradually lose the ability to

transmit the curly-top virus because of physiological changes brought about by old age, or it may simply be depleting its store of virus in feeding as it is transferred daily to a healthy beet. Experiments were performed to determine the ability of the beet leafhopper to transmit curly top during various periods of adult life.

Previously noninfective beet leafhoppers which had spent 10, 30, 40, 60, 70, 75, 90, 100, and 120 days of their adult life on sweet corn were tested for their ability to acquire and transmit curly top. In each test 20 leafhoppers were fed for one day on a curly-top beet and were then

TABLE 7

Ability of Beet Leafhoppers to Acquire and Transmit Curly Top During Various Periods of Adult Life*

Adult age, in days	Adults infective		Adult age, in days	Adults infective		Adult age, in days	Adults infective	
	Number	Per cent		Number	Per cent		Number	Per cent
Experiment No. 1			Experiment No. 2			Experiment No. 3		
10	12	60	10	13	65	10	11	55
40	14	70	30	7	35	30	11	55
70	10	50	60	6	30	60	5	25
100	9	45	90	9	45	75	9	45
						100	2	10
						120	7	35

* The twenty leafhoppers used in each test were fed for 1 day on a curly-top beet and 10 days on a healthy beet.

transferred singly to 20 healthy beets on which they were kept for a period of 10 days. The results of three experiments are presented in table 7.

The data in table 7 show the percentage of leafhoppers which were able to acquire and transmit curly top during various periods of adult life to be as follows: Experiment No. 1, 60 per cent on the 10th day and 45 per cent on the 100th day, experiment No. 2, 65 per cent on the 10th day and 45 per cent on the 90th day, and experiment No. 3, 55 per cent on the 10th day and 35 per cent on the 120th day. These percentages show losses of only 15 to 20 per cent in the ability of leafhoppers to acquire and transmit curly top during later adult life as compared with early adult life. Figure 9 shows the results of the three experiments. This loss is not great enough to explain the loss of the infective capacity by leafhoppers which have not had access to a source of virus for a long period of time.

Figure 10 shows that only 10 to 21 per cent of the leafhoppers fed

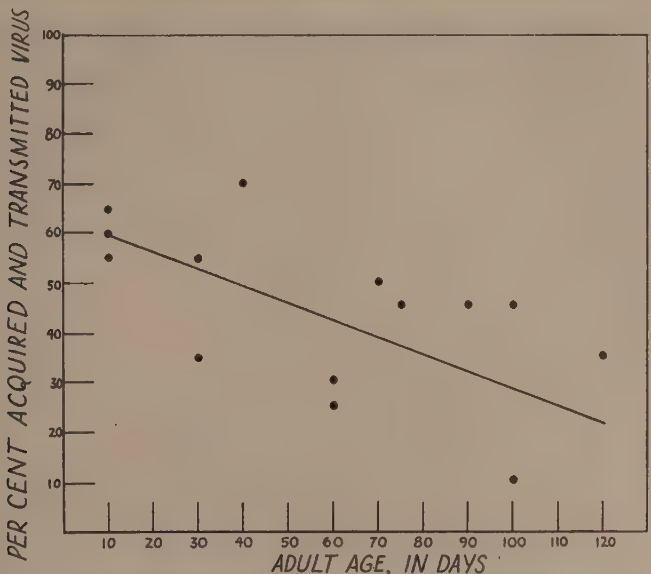

Fig. 9.—Percentage of noninfective beet leafhoppers kept on sweet corn and tested at different ages that acquired the virus by feeding 1 day on a curly-top beet and transmitted it to a healthy beet during the following 10 days.

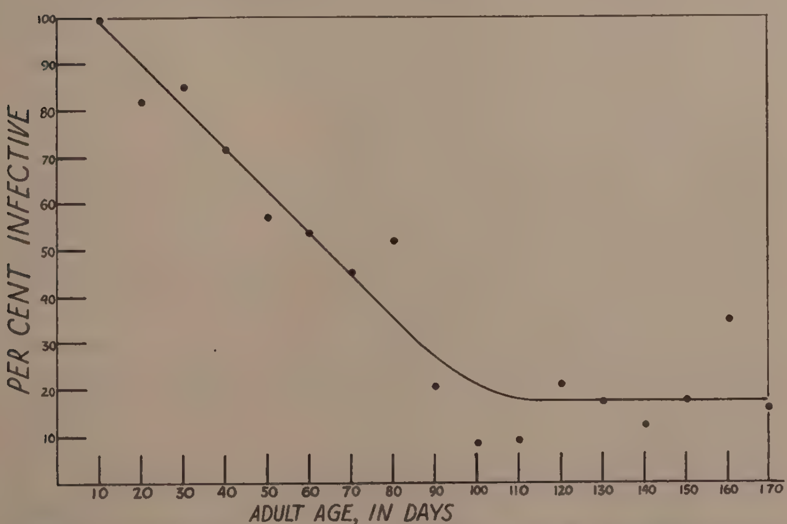

Fig. 10.—Decrease of infective capacity in beet leafhoppers as indicated by the percentage of them which infected beets during successive 10-day periods of adult life. The nymphal stages were completed on diseased beets and the adults were fed daily on a healthy beet during their natural life. (Data from tables 4 and 5.)

during the nymphal stages on diseased beets were still infective from the 100th to 120th day of adult life, as indicated by the percentage of them which infected beets during 10-day periods, when transferred daily to a healthy beet. Since 100 per cent of these insects were infective during the first ten days of adult life, the percentages show a decrease in the infective capacity of 79–90 per cent, which is several times greater than that shown in figure 9 for the loss of the ability to acquire and transmit curly top by previously noninfective leafhoppers. The data in figure 10 were taken from tables 4 and 5.

These data present additional evidence in favor of the theory that infective beet leafhoppers slowly decrease their store of virus and that if they are not reinfected by feeding on a diseased plant to replenish their store of virus, the infective power is gradually lost. Probably the marked decrease in the number of infections by the leafhoppers was only slightly due to physiological changes brought about by the aging of the insect.

REINFECTION OF BEET LEAFHOPPERS WITH CURLY TOP DURING LATER ADULT LIFE

Previous experiments showed that many of the leafhoppers lost the capacity to produce infection during the latter part of their adult life when they were transferred daily to a healthy beet. Two experiments were conducted to determine whether this was due to an exhaustion of the insect's supply of virus or whether the insects had developed an immunity to the virus after having once been infective. Immunity would be indicated by a failure to become reinfected after having once been infective and then having become noninfective.

In the first experiment four recently molted infective leafhoppers that had completed their nymphal stages on a diseased beet were transferred to sweet corn for a period of 40 days, and then each insect was provided with a healthy beet at intervals of 24 hours for 10 days. They were then returned to sweet corn for a period of 43 days, after which they were again transferred daily to successive healthy beets for a period of 53 days. The leafhoppers were reinfected with virus on the 147th to 149th day of their adult life by allowing them to feed on a curly-top beet. After this, the insects were transferred to a healthy beet daily to the end of their natural life.

As is evident from table 8, leafhoppers may be reinfected with curly top during the latter part of their adult life, and apparently do not develop an immunity to the virus after once having been infective. The four insects reinfected by feeding them on a curly-top beet from the 147th to the 149th day of their adult life transmitted curly top to healthy

beets during the latter part of their natural life. As indicated in table 8, male No. 2 infected none of the 10 beets on which it fed during the 41st to 50th day, and also none of the 53 beets during the 94th to 146th day of its adult life. When, however, this male was reinfected by feeding on a curly-top beet for 3 days, it infected 4 of 37 beets on which it fed during the 150th to 186th day of its adult life. Male No. 3 infected only 1 of 10 beets on which it fed from the 41st to 50th day and only 2 of 53 beets during the 94th to 146th day of its adult life. After being reinfected by feeding for a period of 3 days on a curly-top beet, this leafhopper infected 5 of 36 beets on which it fed during the 150th to 185th day of its adult life.

In the second experiment, three infective insects which had completed their nymphal stages on a curly-top beet were confined on sweet corn for 105 days, and then each one was transferred to three successive healthy beets for periods of 14, 7, and 7 days, respectively. They were reinfected by allowing them to feed for 5 days on a curly-top beet from the 134th to 138th day of their adult life. The leafhoppers were then provided with a healthy beet daily for the remainder of their adult life.

The results in table 8 show that the three leafhoppers transmitted curly top to beets after being reinfected with the virus. Female No. 2 infected none of the three beets on each of which it fed for periods of 1 to 2 weeks during the 106th to 133rd day of its adult life. This insect was then reinfected by exposure to a curly-top beet, after which it infected 7 of 20 beets on which it fed during the 139th to 158th day of its adult life. Female No. 3, after 105 days on sweet corn, failed to infect a healthy beet on which it fed for 14 days, but infected 2 successive beets on each of which it was confined for a period of 7 days. This female was reinfected and subsequently infected 16 of 50 beets to which it was exposed during the 139th to 188th day of its adult life.

The results of other experiments would indicate that male No. 2 and females No. 1, No. 2, and No. 4 in table 8 were infective during the early part of their adult life although they produced no infections during the period of time that they were confined on beets. Preceding experiments on the transmission of curly top by insects that had completed their nymphal stages on a diseased beet (tables 4 and 5) show that the 28 leafhoppers were all infective. Figure 5 shows that in one experiment 5 of 12 of these infective insects produced no infection between the 41st and 50th day of their adult life, but all produced curly-top infections during a later period.

The seven leafhoppers in the present experiments probably had a greatly reduced infective capacity before they were reinfected on the

TABLE 8

REINFECTION OF LEAFHOPPERS FED DURING NYMPHAL STAGES ON DISEASED BEETS WITH CURLY TOP DURING LATER ADULT LIFE

Insect sex and No.	Dates on host plant	Host plant	Number of days on host plant	Period of adult life on host plant, days	Adult age when infection produced, days
			Experiment No. 1		
♂ 1	Apr. 9–May 19	Corn	40	1–40	....
	May 19–May 29	Healthy Beet	10*	41–50	41, 42, 43
	May 29–July 11	Corn	43	51–93	....
	July 11–Sept. 2	Healthy beet	53*	94–146	121, 146
	Sept. 2–Sept. 5	Curly-top beet	3	147–149	....
	Sept. 5–Sept. 20	Healthy beet	15*	150–164	157
♂ 2	Apr. 9–May 19	Corn	40	1–40	....
	May 19–May 29	Healthy beet	10*	41–50	....
	May 29–July 11	Corn	43	51–93	....
	July 11–Sept. 2	Healthy beet	53*	94–146	....
	Sept. 2–Sept. 5	Curly-top beet	3	147–149	....
	Sept. 5–Oct. 12	Healthy beet	37*	150–186	156, 161, 165, 185
♂ 3	Apr. 9–May 19	Corn	40	1–40	....
	May 19–May 29	Healthy beet	10*	41–50	42
	May 29–July 11	Corn	43	51–93	....
	July 11–Sept. 2	Healthy beet	53*	94–146	103, 115
	Sept. 2–Sept. 5	Curly-top beet	3	147–149	....
	Sept. 5–Oct. 11	Healthy beet	36*	150–185	155, 159, 172, 174, 179
♀ 1	Apr. 9–May 19	Corn	40	1–40	....
	May 19–May 29	Healthy beet	10*	41–50	....
	May 29–July 11	Corn	43	51–93	....
	July 11–Sept. 2	Healthy beet	53*	94–146	....
	Sept. 2–Sept. 5	Curly-top beet	3	147–149	....
	Sept. 5–Oct. 11	Healthy beet	36*	150–185	154, 184
			Experiment No. 2		
♀ 2	July 25–Nov. 7	Corn	105	1–105	....
	Nov. 7–Nov. 21	Healthy beet	14	106–119	....
	Nov. 21–Nov. 28	Healthy beet	7	120–126	....
	Nov. 28–Dec. 6	Healthy beet	7	127–133	....
	Dec. 6–Dec. 11	Curly-top beet	5	134–138	....
	Dec. 11–Dec. 31	Healthy beet	20*	139–158	140, 141, 142, 151, 153, 154, 157
♀ 3	July 25–Nov. 7	Corn	105	1–105	....
	Nov. 7–Nov. 21	Healthy beet	14	106–119	....
	Nov. 21–Nov. 28	Healthy beet	7	120–126	120–126
	Nov. 28–Dec. 6	Healthy beet	7	127–133	127–133
	Dec. 6–Dec. 11	Curly-top beet	5	134–138	....
	Dec. 11–Jan. 30	Healthy beet	50*	139–188	140, 145, 147, 148, 150, 152, 154, 159, 161, 164, 166, 169, 173, 175, 178, 187
♀ 4	July 25–Nov. 7	Corn	105	1–105	....
	Nov. 7–Nov. 21	Healthy beet	14	106–119	....
	Nov. 21–Nov. 28	Healthy beet	7	120–126	....
	Nov. 28–Dec. 6	Healthy beet	7	127–133	....
	Dec. 6–Dec. 11	Curly-top beet	5	134–138	....
	Dec. 11–Jan. 10	Healthy beet	30*	139–168	154

* Leafhopper transferred to healthy beet daily for number of days indicated.

134th and 147th day of their adult life. As shown in figure 5, the six leafhoppers still alive after the 130th day produced only one infection, although each insect was exposed to from 14 to 76 beets, or an average of 48 beets. The results presented in table 8 show that the 7 insects in the two experiments which were reinfected, produced from 1 to 16 infections, or an average of 4.6 infections, while feeding on from 15 to 50 beets, or an average of 32 beets, during their later adult life. The evidence clearly indicates that the beet leafhopper can be reinfected with the curly-top virus.

INCUBATION PERIOD OF DISEASE IN SUGAR BEETS

Leafhoppers with a greater infective capacity might inoculate more virus into the plant during their feeding and for that reason there might be a shorter incubation period of the disease in the beets. In order to test this hypothesis a comparison was made of the incubation period of the disease infected by adults fed for periods of from 10 minutes to 3 hours on curly-top beets, with those infected by adults which had completed their nymphal stages on a source of virus.

The results, summarized in table 9, show that the incubation period of the disease in the first beet infected by previously noninfective adults which had fed for periods of from 10 minutes to 3 hours on a diseased beet varied from 6 to 35 days. The incubation period of the disease in the first beet infected by leafhoppers fed during all of their nymphal stages on diseased beets varied from 6 to 35 days (table 10). These results indicate no apparent differences in the length of the incubation period of the disease in the first beet infected by adults fed for varying periods on a diseased plant.

The results of table 9 indicate that the incubation period of the disease in successive infections by leafhoppers which had fed for only short periods during their adult life on a diseased beet varied from 5 to 39 days, while this period varied from 5 to 38 days with adults which completed the nymphal stages on diseased beets (table 10). The fluctuations were irregular, and the occasional prolonged periods are difficult to explain. The variation may be attributable to slight differences in size or resistance of the beets or in growing conditions.

It seems possible that, as the virus becomes more dilute in the insect with successive feedings on healthy plants, the incubation period of the disease in the beets would be increased. The minute quantity of virus injected into the feeding punctures by such insects as they slowly exhausted their store of virus would take a long time to multiply and cause symptoms of the disease to develop. The evidence obtained, however,

TABLE 9

Incubation Period of the Disease in Beets Infected by Beet Leafhoppers Fed Short Periods on Curly-Top Beets and Then Provided with a Healthy Beet Daily During Adult Life

Time on curly-top beets, minutes	Insect sex and No.	Adult age when infection produced, days	Incubation period of disease in beets, days
10	♀ 1	36	11
	♀ 3	15	27
		50	19
	♀ 4	9	35
		17	5
		18	13
		32	15
	♂ 1	1	9
		3	8
		9	8
		31	8
	♂ 2	5	12
		48	39
		81	34
20	♀ 5	7	14
		43	7
		45	7
	♀ 6	1	17
	♂ 3	15	6
	♂ 4	1	14
		9	11
		13	11
		14	12
		25	7
		71	26
	♂ 5	7	11
		117	17
40	♀ 7	7	11
	♀ 8	10	12
		115	10
		135	*
		161	*
	♂ 6	5	32
		16	7
		18	7
		29	32
		34	8
	♂ 7	8	9
		17	19
60	♀ 9	10	16
		12	15
		18	18
	♀ 10	7	10
		12	11
		18	37
	♀ 11	4	20
		21	*
	♀ 12	7	7
		17	18
		25	13
		102	*
		107	9
		123	17
		130	21
	♂ 8	8	13
		10	5
		16	15
		23	11
		68	16
120	♀ 13	9	35
		11	6
		35	8
		42	23
		68	14
		77	9
	♂ 9	3	12
		9	14
		11	10
		16	15
		25	13
180	♂ 10	1	11
	♂ 11	9	8
		10	6
		32	5
	♂ 12	9	9
		13	8
		27	*
		29	17
		36	8
		42	13
		52	12
		67	*
		105	*

* No records kept.

TABLE 10

INCUBATION PERIOD OF DISEASE IN BEETS INFECTED BY BEET LEAFHOPPERS FED DURING NYMPHAL STAGES ON CURLY-TOP BEETS AND THEN PROVIDED WITH A HEALTHY BEET DAILY DURING ADULT LIFE

Insect sex and No.	Adult age when infection produced, days	Incubation period of disease in beets, days
♀ 1	2	35
	6	18
	7	6
	8	35
	9	21
	13	14
	16	8
	29	16
	38	13
	50	38
♀ 2	6	10
	8	16
	9	11
	10	11
	13	6
	18	7
	20	15
	22	7
	26	17
	28	10
	29	23
	30	10
	31	14
	33	14
	34	15
	38	9
	42	9
	44	11
	45	8
	46	17
	56	14
	83	17
♀ 3	5	14
	6	7
	7	8
	9	5
	16	12
	25	12
	26	7
	39	19
	40	11
	55	13
	59	25
♀ 4	2	17
	7	10
	33	8
	80	32
♀ 5	3	9
	5	15
	6	25
	7	*
	20	17
	25	7
	33	7
	48	8
	49	9
	64	25
	73	31
	87	10
♀ 6	1	24
	2	7
	3	13
	4	7
	5	12
	8	6
	9	11
	10	14
	13	11
	15	6
	19	8
	20	13
	22	9
	27	11
	28	9
	29	8
	31	*
	35	12
	36	15
	56	39
	65	19
	67	9
♀ 7	2	16
	5	21
	6	10
	7	11
	9	16
	10	9
	12	12
	14	9
	15	7
	16	16
	19	8
	22	6
	24	6
	26	7
	30	*
	35	21
	48	18
	50	9

* No records kept.

Table 10 concluded on page 338.

TABLE 10 —(*Concluded*)

Insect sex and No.	Adult age when infection produced, days	Incubation period of disease in beets, days	Insect sex and No.	Adult age when infection produced, days	Incubation period of disease in beets, days
	1	15		1	7
	2	27		2	9
	8	8		3	7
	15	8		6	21
	22	5		8	13
	23	5		9	7
	27	17		11	8
	30	9		12	11
♀ 8	37	6		13	8
	60	*		16	10
	63	7		21	10
	64	10		23	14
	76	11	♂ 1	24	12
	77	5		26	10
	80	8		27	10
	82	7		30	13
	108	17		32	15
				33	7
	4	8		39	13
	5	7		43	13
	21	*		44	10
	29	8		48	6
	31	13		49	15
	42	29		51	9
	43	15		58	9
♀ 9	45	15		59	7
	49	11			
	50	13		2	6
	53	22		4	13
	54	9		5	12
	57	10		7	18
	100	26		9	12
	104	23		10	11
				11	9
	1	20		12	13
	2	13		13	18
	3	6		14	8
♀ 10	4	9	♂ 2	16	8
	6	10		17	7
	22	10		24	14
	68	31		27	11
	151	13		29	7
				31	7
				33	7
				39	9
				42	14
				47	7
				52	*
				78	33

* No records kept.

does not indicate a lengthening of the incubation period of the disease as the infective capacity of the insect is progressively decreased: the period in the last beet infected was usually no longer than for infections produced during the early adult life of the leafhopper.

SUMMARY

An investigation was undertaken to determine whether the curly-top virus of the sugar beet multiplied in the beet leafhopper. If a multiplication occurs in the leafhopper, the insects probably should not only retain the infective capacity during their entire adult life, but those fed for only a short period on a diseased beet should also be able to cause as many infections as those fed for long periods. Accordingly, experiments were conducted in order to determine whether the infectivity of the insects remained constant during their adult life and whether the length of the feeding period on a diseased beet modified the infective capacity of the insects. Leafhoppers were fed for varying periods on curly-top beets and tested for their infectivity by transferring them daily to healthy beets. The daily transfers were considered frequent enough to prevent the insect from reinfecting itself with virus after infecting the healthy beet.

The results of the investigation indicate that the curly-top virus does not multiply in the beet leafhopper. No evidence was found to support such a theory as has often been surmised on the basis of indirect evidence.

There was a gradual decrease in the percentage of beets infected by the leafhopper during successive 30-day periods of adult life when the insects were transferred daily to a healthy beet. When adults or nymphs were fed for longer periods on diseased beets, they remained highly infective for a longer period than those which had fed short periods. The distribution of the infections caused by the beet leafhopper during its adult life failed to indicate a multiplication of the virus in the insect.

Many of the infective leafhoppers apparently lost the capacity to produce infection during late adult life; others retained their infectivity but infected beets only at great intervals. Insects which had completed the nymphal stages on curly-top beets were confined as adults on curly-top-immune sweet corn and were tested every 10th day of adult life for their capacity to transmit curly top. These insects showed a gradual loss from 50.0 per cent transmission on the 1st day to 3.1 per cent on the 90th day. If permanence of infective power during the life of the insect is the basis for the assumption that a multiplication of the virus occurs in the vector, then it cannot be argued that a multiplication of the curly-top virus occurs in the beet leafhopper.

A rather strong point against the theory that a multiplication of the curly-top virus occurs in the leafhopper was found in the comparative study of the number of infections produced by insects after short and long feeding periods on diseased beets. Insects which had fed only a short period of time on a curly-top beet were capable of producing only an average of 3.4 infections when transferred daily to successive healthy beets during adult life, while insects fed for long periods caused an average of 15.6 infections. Leafhoppers which had fed for short periods on curly-top beets probably accumulated less virus and consequently produced fewer infections than those which fed for long periods; these facts indicate that the leafhoppers are merely internal mechanical carriers.

Experiments were conducted to test the possibility that the infrequent transmissions during late adult life of the insect may have been due to the inability of old insects to transmit the disease readily. These experiments showed that leafhoppers first infected during the latter part of their adult life do not transmit the disease as frequently as those infected early in their lives. However, this decrease in infectivity is less than that which occurs in insects infected during early adult life and kept on healthy plants until the latter part of their lives. Some leafhoppers reinfected with the virus by feeding on a curly-top beet during later adult life transmitted the virus as readily as recently molted adults. The evidence shows that the beet leafhopper does not lose the ability to acquire or transmit the virus as it approaches old age and that the insect can be reinfected with the virus during late adult life.

While carrying out the experiments to determine the infective capacity of insects fed for short periods on diseased beets, great variation was found in the length of the period of delay in the development of the infective capacity. The "incubation period" or the period of time that elapsed between the initial feeding of from 10 minutes to 3 hours on a curly-top beet and the first infection by the leafhopper varied from 1 to 44 days, or an average of 9.6 days. The insects produced infections at very irregular intervals after the first infection. This result does not suggest a multiplication, since one would expect to get infections at regular intervals if there was an increase of virus in the insect.

A comparison of the transmission of curly top by male and female leafhoppers revealed no differences that could be considered significant in view of the great variation within each sex.

There was no significant difference in the length of the incubation period of the disease in beets infected by leafhoppers fed for varying periods on curly-top beets.

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